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PROCEEDINGS OF A CONFERENCE
ON
COTTONSEED
PROTEIN CONCENTRATES

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~~Held at~~ New Orleans, ~~Louisiana~~
January ~~15-17~~, 1964

United States Department of Agriculture
Agricultural Research Service

ABBREVIATIONS USED IN THIS REPORT

UNICEF	United Nations Children's Fund
WHO	World Health Organization
ARS	Agricultural Research Service
HEW	Health Education and Welfare
FDA	Food and Drug Administration
MIT	Massachusetts Institute of Technology
INCAP	Institute of Nutrition of Central America and Panama
SURDD	Southern Utilization Research and Development Division
BLS	Bureau of Labor Statistics
FAO	Food and Agriculture Organization of the United Nations
NCPA	National Cottonseed Products Association

In most articles in this report underscored numbers in parentheses refer to literature cited at the end of the article. The artwork and tables are reproduced essentially as they were supplied by the writer of each paper.

FOREWORD

Increasing interest in improving the nutritive quality of our diet and efforts to supply protein to large areas of the world have focused attention on oilseed protein concentrates.

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~~This conference~~ was sponsored jointly by USDA's Agricultural Research Service, and Oth United Nations Children's Fund (UNICEF), and the National Cottonseed Products Association to foster that interest and further the efforts by bringing together leaders in this field of research to review technological progress that has been made in cottonseed processing, and to try to project the direction of future research in this area. It was attended by over 140 persons, representing the cottonseed, food and associated industires, Federal, State and foreign research laboratories, and international agencies, as well as staff members of the Southern Utilization Research and Development Division.

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~~These proceedings,~~ which were compiled by Beatrice A. Sharar of this ~~Division~~, report the statements presented by the various speakers during the conference and give an account of the discussion which followed.

C. H. Fisher
Director, Southern Utilization Research and Development Division

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CONFERENCE ON COTTONSEED PROTEIN CONCENTRATES

Introduction

The Conference on Cottonseed Protein Concentrates was called to order by General Chairman Aaron M. Altschul. Dr. Altschul introduced Dr. Bruno H. Wojcik, Laurence W. Mazzeno, Jr., and Beatrice A. Sharar as some of those directing the conference. He then introduced Dr. C. H. Fisher, Director of the Southern Utilization Research and Development Division.

WELCOME

by
C. H. Fisher
Director
Southern Utilization Research and
Development Division
New Orleans, Louisiana

It is a real honor and privilege as Director of the Southern Division to welcome this splendid audience to this International Conference on Cottonseed Protein Concentrates. Many of you have traveled considerable distances to be here, and some of you have made even longer trips and have come from other countries.

We are glad all of you are here. We wish to give special welcome and warm greetings to our visitors from other countries.

As you are aware, this international conference is sponsored jointly by UNICEF, the National Cottonseed Products Association and USDA's Agricultural Research Service as represented by the Southern Utilization Research and Development Division. The Southern Division is grateful for the privilege of being a cosponsor. We are grateful to UNICEF for their important assistance in facilitating the attendance of some of our visitors.

We in the Southern Division are particularly pleased to cosponsor a conference dedicated to the memory of the late H. D. Fincher. We loved "Doc" Fincher as a good and true friend. We admired him for his excellent personal qualities and his devotion to research and his fellowman. We are very grateful because we had the benefit of his excellent technical advice for many years. "Doc" Fincher did much to advance the science of cottonseed processing and it is indeed fitting that we dedicate this conference to his memory.

I wish to express gratitude to the many persons contributing to this conference, including Max Milner, Layton Allen, Garlon A. Harper, and Richard A. Phelps of UNICEF and NCPA. We are deeply grateful also to our speakers, chairmen, discussion leaders, and to the Program Committee, Aaron M. Altschul, Layton E. Allen, Garlon A. Harper, Vernon L. Frampton, Leo A. Goldblatt, and Henry L. E. Vix. Dr. B. H. Wojcik and members of his staff planned the conference and supervised the arrangements. We thank Dr. Wojcik, L. W. Mazzeno, Jr., Beatrice A. Sharar, Mary Alice Jones, and Shirley Saucier for their good work on behalf of the conference.

I think we should give special mention to the Chairman of the Program Committee, Dr. Aaron M. Altschul: It has been his foresight, enthusiasm and competent work that have been primarily responsible not only for this conference, but also for the successful protein conference held about one year ago. It is appropriate to mention also that our Seed Protein Pioneering Laboratory, under the imaginative leadership of Dr. Altschul, has made important advances to our knowledge of cottonseed and other oilseeds.

As a concluding remark, I wish to comment upon the tremendous importance of the subject of our conference. Cottonseed and its products are of great economic and scientific importance in the United States and in many other countries. The subject of our international conference also has the potential and promise of providing better nutrition, health and life for millions of human beings in many parts of the world.

You will note from the program that at this point Dr. George W. Irving, Jr., Deputy Administrator, ARS, Washington is scheduled to make remarks. Dr. Irving is a very busy man. In addition to being responsible for the four Utilization Divisions, Dr. Irving administers the three divisions engaged in human nutrition and consumer use research. The many requirements of this position combined with last minute emergencies made it impossible for him to be with us, but he sent regrets and better than that, he sent Dr. Ruth Leverton, Assistant Administrator of ARS to represent him. Dr. Leverton.

REMARKS

by

Ruth M. Leverton
Assistant Administrator
Agricultural Research Service
Washington, D. C.

Thank you, Dr. Fisher and good morning all. I am sure that you have all had the experience of substituting for people. It isn't always that when you do you have a speech already written for you. But because I have had this experience a good many times, it behooves me to find a fairly good story that takes the curse off being up here when someone else was wanted.

There is the story of a young minister who at the last minute was called in to substitute in the pulpit when the regular minister became ill. When the young minister got up to address his congregation, he said, "I am here as a substitute for your regular clergyman. You know what a substitute is. Look at these lovely windows, the beautiful colors of glass and all. Now, if one of these panes were broken, and you put a piece of cardboard up there, that would be a substitute, and that is what I am this morning."

So he went forth with his sermon and did the very best he could. And at the end of the service, one of the leading members of the congregation came up to him and said, "Oh, Reverend, you weren't a substitute. You were a real pane."

Dr. Irving is indeed sorry that he could not be here and these are the things that he would like to have said to you on this occasion.

OPENING REMARKS

by

George W. Irving, Jr.
Deputy Administrator
Agricultural Research Service
Washington, D. C.

(Presented by Ruth M. Leverton)

To point out that this is an age dominated by science and technology may be a cliche, nevertheless it is a true statement, There is hardly a facet of human life which is not touched by new research developments in science and technology; and almost invariably the results are for the better. Just one example is how our ability to feed people has been expanded and extended by improvements in science and technology. Yields of food per acre and efficiency of production of animals have increased while the quality of these food products has been improved and modified.

This meeting is an illustration of the significant advances in the field of food science. The story of cottonseed protein is the story of ever-increasing usefulness through application of new information and improved processing. When cottonseed was first available, the entire seed was considered a nuisance, creating only a disposal problem. When the oil was recognized as economically valuable, the residue still was considered a waste product suitable only for fertilizer. Then came the extensive use of cottonseed protein concentrates for ruminant animals, but for a long time this was not considered suitable for nonruminants. The Southern Division, in cooperation with the National Cottonseed Products Association andwith the various State Experiment Stations, undertook an extensive program of research to understand better the role of composition and processing techniques on the value and usefulness of cottonseed protein. This research was interspersed with a number of meetings to bring together the investigators in the field and to exchange information. Results of the research made clear that cottonseed meal could be manufactured in such a way as to be suitable for supporting growth of monogastric animals, as well as ruminants. Now cottonseed meal has a firm place in poultry feeds and in many instances it is interchangeable with large quantities of soybean protein concentrate when the economics favor such an interchange. There are still problems to be solved in producing the best quality of protein concentrate from cottonseed; many of these problems no doubt will be discussed at this meeting.

The success in making available another large source of plant protein for monogastric animals led to the challenge of making a similar product suitable for human consumption. A small commercial operation to produce cottonseed protein concentrate for human food had existed in the United States for some time. In cooperation with United Nations agencies and with INCAP in Guatemala, members of the staff of this Division made it possible to test cottonseed protein concentrates in human foods, and then assisted in setting up standards and developing conditions for commercial production of such concentrates.

At the first meeting on cottonseed protein concentrate held over 3 years ago we heard the exciting story of the beginnings of Incaparina and attempts in other parts of the world to use cottonseed protein concentrate directly in human foods. We are now meeting again to evaluate progress since that time, both in the introduction of cottonseed protein products in human food and in developing procedures for producing improved products. At this international meeting scientists and technologists from many parts of the world will have the opportunity to trade information and to set the basis for further gains in broadening the utilization of proteins from cottonseed.

The U. S. Department of Agriculture is proud to have played so important a role in bringing cottonseed protein utilization to this point. We are happy that we had the good fortune to work together with scientists in industry, with the National Cottonseed Products Association, and with scientists in many of the State Agricultural Experiment Stations. We are continuing our research to obtain further information on the composition and properties of cottonseed and cottonseed protein products and to develop new methods for processing. Our fundamental research program on cottonseed has been strengthened recently by investigations on the ultrastructure and biochemistry of cottonseed.

We are grateful to UNICEF and the National Cottonseed Products Association for joining with us in this meeting. You can rest assured that Dr. Fisher and his staff, and all of us in the Department, are very much interested in this program. We are looking forward to continued and increased cooperation with all of you to the end of making sure that the utilization of this great potential source of protein is expanded, not only in the United States but wherever in the world the technique and facilities are available for producing quality products.

OPENING REMARKS

by

Grace A. Goldsmith
Professor of Medicine
Tulane University
New Orleans, Louisiana

It is a pleasure to participate in the opening session of this Conference on Cottonseed Protein Concentrates. The importance of developing new sources of good quality protein that can be used to improve the nutritive quality of diets in many parts of the world is widely recognized. Malnutrition, and particularly protein malnutrition, is one of the most serious problems in the world today especially in the developing countries. Infants and young children are the most vulnerable group and mortality and morbidity is high. Malnutrition affects not only physical well being but also mental and emotional health. Productivity is decreased and the whole economy suffers. It is obvious that the nutrition and health needs of a country's population should have top priority.

Many national and international groups are working together in an attempt to solve the problem of providing an adequate food supply for a rapidly expanding world population. The program of the Food and Agriculture Organization of the United Nations and the Freedom from Hunger Campaign are well known. The Protein Advisory Group and the Joint Expert Committee on Protein Requirements of FAO and WHO are making contributions in this area. UNICEF, one of the cosponsors of this conference, is extremely active in this field. The Food and Nutrition Board of the National Academy of Sciences-National Research Council has been interested for a number of years in the widespread problem of protein deficiency. Through a special Committee on Protein Malnutrition, it has initiated and guided a research program, under a grant from the Rockefeller Foundation, to develop protein-rich foods which can be used in the prevention and treatment of protein malnutrition. The foods chosen must be inexpensive, locally available and acceptable to the population in question. Studies have been carried out in many areas of the world and considerable progress has been made. Various combinations of vegetable protein have been found to be most efficacious. Cottonseed protein concentrate, the subject of today's program, is one of the valuable dietary supplements.

Last spring, the Committee on Protein Malnutrition held a working conference to consider methods for evaluation of protein quality. An international group of twenty participants deliberated for several days and prepared a report and recommendations. This is now available and may be of interest to participants in this symposium.

The Food and Nutrition Board and the American Institute of Nutrition have great interest in strengthening nutritional aspects of international programs. In 1961, the Board published a pamphlet on the Role of Nutrition in International Programs and in 1963 another statement was formulated entitled "Recommendations on Administrative Policies for International Food and Nutrition Programs." In the latter report, the need for effective action programs to meet urgent food and nutritional deficiencies in many developing areas of the world was stressed and the importance of food technology in this program was emphasized. The Board urged that the United States institute a comprehensive, sustained, and coordinated program in nutrition and food technology including related aspects of agriculture, industry, education, and public health. Attention should be given to food production, processing, distribution, and sanitation as well as to support of research and the training of personnel. It was hoped that such a program might be supported by the Agency for International Development or some other authorized Federal Agency to meet the critical need. It was suggested that a top level executive be appointed with sufficient responsibility to ensure coordination of international activities of all government departments having a major interest in nutrition and food technology. The Board felt that a primary objective of foreign policy of the United States as it relates to food and nutrition should be prevention of serious malnutrition among children.

A conference such as the one beginning today, sponsored jointly by the Agricultural Research Service of the United States Department of Agriculture, UNICEF, and the National Cottonseed Products Association, can make a significant contribution to knowledge of an important protein supplement which will be most useful in combating malnutrition. Conferences such as this, by bringing together leaders in a specific area of research, stimulate interest, understanding and cooperation among diverse groups. Technological progress is reviewed, unsolved problems are pointed out, and potential practical applications of present knowledge are suggested. These deliberations should assist in developing more effective programs to improve nutrition and health of people throughout the world.

DEDICATION

by

Aaron M. Altschul
Southern Utilization Research and Development Division
New Orleans, Louisiana

It has fallen upon me to carry out the wishes of the Program Committee to make the dedication at this conference to the memory of H. D. Fincher, known to his many friends as "Doc", who passed away May 14, 1963. I welcome this opportunity to pay tribute to the memory of a close personal friend and a colleague of long standing.

Doc graduated from Georgia Tech in 1927 and spent 17 years with Procter and Gamble, Oil Milling Operations, after which he joined the staff of Anderson Clayton Co., and was associated with them for the rest of his career. He was thoroughly familiar with every aspect of oilseed processing. His job was to troubleshoot, to solve problems, to accomodate the operations of this industry to changing times and changing opportunities, and to develop and put into operation new processes. It was during his career that the cottonseed processing industry changed its operations drastically from mechanical pressing to solvent extraction, or to combinations of mechanical pressing and solvent extraction. He had great responsibility for effecting these changes; it is because of people like him that the cottonseed processing is a modern industry capable of turning out quality products and prepared to improve its operations through its accumulated technical know-how.

A great deal of this technical know-how was accumulated in the person of Fincher himself. Even if all of his contributions were within his own company, this in itself would have made him a pioneer in this period of transition in oil milling operations. But, he did not confine his efforts to strictly company interest. He served actively on the Research Committee of the National Cottonseed Products Association and was chairman of several of its subcommittees. He was active in the planning of conferences within the industry, or between Government and academic research institutions and industry. He was an adviser to the Southern Division on its research program. When UNICEF needed industrial help for programs to manufacture oilseed protein concentrates suitable for human food, one of the first whose help was sought, and was freely given, was that of H. D. Fincher. When I needed a man to write an authoritative chapter on oilseed processing for my book I called upon Doc. His chapter stands as one of the best descriptions of oilseed processing in the literature.

If I had to choose two qualities of Doc for emphasis, I would choose his competence and his modesty. He knew oilseed milling; he knew oilseed processing; he knew all the facets and he knew where to get the information. He was a credit to his organization and his associates, and was a credit to American industry. He was a modest man. He was never self-seeking, he was comfortable to be with. It was always a pleasure to see him; it was always a pleasure to discuss things with him; no one had to worry about how he stood and how his feelings would react to this or that demand on his time or his energy.

His fame was not confined to this country. It was my sad duty to inform those who were planning the UNICEF/FAO meeting on protein concentrates in Rome last October, that Fincher had passed away. And I had to perform the same duty when I informed the planners of the forthcoming oilseed protein conference in Japan, that Fincher, on whom they had counted for an important part of the program, had passed away.

When we planned this meeting, we had in mind his active participation and counsel, and we will sorely miss him. The next best thing we can do is to enter into this meeting with the spirit that dominated his own activities, to seek the best understanding of the problems and the operations that we are trying to deal with, not to be afraid of making changes, rather to welcome the challenges which come as we try to realize more of the potential for human well being inherent in our natural resources.

The audience rose a moment in silent tribute.

H. D. Fincher

REMARKS

by

Layton E. Allen

UNICEF

United Nations, New York

On behalf of UNICEF I wish to thank all of you for participating in this conference. We believe it will be a good one and we will learn from each other.

UNICEF is represented here by three senior members of the Food Conservation Division, Mr. Hill, Dr. Milner, and myself. The Food and Agriculture Organization, one of our sister agencies in the United Nations, is represented by Dr. Kapsiotis from the Nutrition Division in Rome, and Dr. Contesse of Peru. The Secretary of the Protein Advisory Group to UN Agencies, Dr. DeMaeyer, is also present. You might ask what is UNICEF and what is its interest in cottonseed?

UNICEF can best be described as a simple organization, operating simple projects for the benefit of simple people.

UNICEF is the autonomous agency within the U. N. that is primarily interested in the health and welfare of the children in the developing countries. The annual budget is about 40 million dollars. Approximately one-sixth of these funds are used in short range and long range nutrition programs. A typical short range program in nutrition is the distribution of skim and whole milk powder provided from Canadian and U. S. surpluses, and UNICEF distributes one-sixth of the U. S. surplus milk powder. A typical long range program would involve the construction of a food processing plant to promote the development and utilization of local foods such as cow and buffalo milk. At present 180 such plants are in operation and 30 are under construction. Another long range program would be the development and introduction of suitable new foods utilizing local high protein materials not normally consumed as foods. Such a project using peanut press cake is underway now for India.

One of the better proteins which is abundant in several of the developing countries and which is being consumed only in minor quantities as a food is the protein of the cottonseed. UNICEF is extremely interested in the development and introduction of new foods using cottonseed protein. The contribution which the participants of this conference are making in the development of the cottonseed protein will enable UNICEF to push ahead on the use of cottonseed protein in the feeding programs. For that important reason, UNICEF is pleased to join the NCPA and USDA in sponsoring this conference.

PROBLEMS AND OPPORTUNITIES IN UTILIZING COTTONSEED PROTEINS TO MEET WORLD PROTEIN DEFICITS

by

Max Milner
UNICEF
United Nations, New York

The growing dietary scarcity of protein in many developing countries, particularly where increasing population pressure is bearing hard on restricted agricultural potential, is a situation becoming widely known to laymen and specialists alike. The very fact that this conference is considering a number of the subjects listed in the program, attests to the feeling of urgency toward this problem among scientists whose work is related to agricultural and food products, and to a growing conviction among them that conventional agriculture, conventional food crops, and traditional food processing may not provide adequate solutions to the world's increasing food needs.

The challenge of this problem to the United Nations prompted initiation some 10 years ago of the so-called WHO/FAO/UNICEF Protein-Rich Foods Program, whereby the agencies proposed to provide international coordination for the dissemination and application of scientific and technical knowledge relating to new or unused sources of protein, and also to undertake projects of assistance to governments requiring help in the processing and use-promotion of such foods. From the vantage point of only several years of experience in this program, it is clear that there was much naivete at that time in the assumption that available technology might be sufficient to initiate effective programs of introduction, processing and utilization.

Attention was focused from the beginning on the oilseed meals and presscakes which have been largely unused as human food. In surveying the potential of these materials as protein resources it became clear that while soybeans are the world's first oilseed crop, amounting to something over 30 million tons of annual production, almost two-thirds of this total (which includes an estimate of Chinese production) is grown in the United States. Thus, except for the major quantity grown also in mainland China, soy is otherwise not widely produced. One drops quickly down the quantity scale to the next production levels, with Brazil at 250,000 metric tons and Indonesia producing 440,000 tons in 1960. In spite of the widely known traditional Asian use of soy protein foods, this crop is grown in only a few developing countries having urgent need for protein.

The FAO production statistics reveal clearly that cottonseed is the most widely distributed oilseed in tropical and subtropical areas, with India, Brazil, Pakistan, Egypt, and Mexico as major producers. Peanut follows closely in quantity and prevalence. Dr. Altschul has pointed out that in terms of quantity, the yield of oilseed protein throughout the world is comparable with the world's production of edible animal protein foods. Only a fraction of the oilseed protein is used, however, for food or feed.

The possibility that crude unsanitary byproduct materials traditionally disposed of as fuel or manure, could be prepared as human food was apparently not generally considered until these recent times. Certainly it is clear from even superficial speculation that the human race in the millenia of its painfully pragmatic and empirical approach to food selection from the flora and fauna of its environment chose, probably wisely on the basis of unhappy experience, to reject as foods these crude residues of oilseed crushing. In fact while there has been edible use of soybeans processed by the complicated empirical methods of Eastern and Southern Asia, in general oilseed residues have at best, and only in recent years, been used to some extent for animal feeding. In India today, nevertheless, most of the peanut presscake (3, 000, 000 tons per annum) is used for manure. Whatever cottonseed is used in India for cattle, is fed almost entirely as the whole seed.

In spite of existing scientific knowledge of the value of oilseed residues for animal feeding, one of the first orders of business required in the international programs was a comprehensive clinically supervised evaluation of their usefulness and safety for human infants. It is noteworthy that many of the most recent efforts to use these products as food were instigated by nutrition scientists, and that food technology and related skills which are usually invoked in countries such as the United States to develop and process new foods prior to testing and marketing in palatable and acceptable form, have not yet become adequately involved. Thus Dr. Scrimshaw and his coworkers with their cottonseed flour mixtures, Subrahmanyan and his peanut products, and Nicol with skim milk supplemented groundnut flour--these and other recent efforts to utilize oilseed residues as economical protein concentrates were motivated largely by urgent world-wide nutritional needs rather than by a desire to open markets for new food products. Our colleagues in the nutrition field have blazed the way and they are urging other food scientists to lend their skills to this pioneering work.

The unsophisticated conviction that nutritional quality and feeding safety of oilseed protein concentrates and fish flours were alone sufficient reasons to initiate acceptability programs, dominated our thinking as a matter of fact until recently. UNICEF has provided commercial U. S. cottonseed flour of edible grade to several developing countries, having large domestic production of cotton, hoping that its nutritional virtues would be quickly recognized and that it would be incorporated and used in local foods on a wide scale. I regret to report that in virtually every instance these efforts are now dormant. This has been due to many reasons, including lack of understanding of the value of this new food, lack of local experience in food formulation and recipe development, and popular resistance to changes in traditional foods. In this context I am looking forward to the report of our visitors from South Africa, where a straightforward commercial approach to introduction, promotion, and marketing of a new protein-rich food is apparently succeeding well. I believe you will be interested to know that FAO and UNICEF are fully aware of the major role which the commercial food industry must play in the production and marketing of protein-rich foods in developing countries. This conviction was implemented last October in Rome at the first international meeting with food industries convened by FAO and UNICEF, which dealt exhaustively with current problems in the production, processing, quality control, packaging, marketing and promotion of new protein-rich foods.

A striking illustration of problems that arise when attempts are made to introduce strange foods, or even to recommend new uses for traditional foods, emerges from our experience with soy products. In spite of the fact that these have been used in Southeast Asia for centuries, we discovered that the traditional foods favored by these populations including "milks" are only infrequently fed to infants. And so today UNICEF finds itself in the interesting position of helping Chinese pediatricians to convince themselves that certain heat-processed soy products can be used safely for infant feeding.

But back to cottonseed--we in UNICEF are now convinced that much unfinished technological business remains to be tidied up before this tremendously valuable protein resource can be utilized to the extent that it deserves. We simply do not have enough technical knowledge of processing and food use to provide the flexible alternatives needed to carry out really sustained programs. I am sure that Dr. Scrimshaw and his colleagues would now agree that had consistently uniform and satisfactory edible cottonseed flour existed in the Central Americas, the commercial Incaparina program might be even farther advanced than it is today. We are of course convinced of the nutritional usefulness of edible-grade cottonseed flours which can be produced by careful manipulation of traditional oil extraction methods, nevertheless we also believe that the commercial products now available, whether in Central America, Colombia or the United States, represent compromises in technology and quality which must be clearly recognized.

What is the nature of this compromise and of the unfinished technological business with cottonseed flour? Before presenting our views on this problem may I emphasize that my colleagues and I have had little direct professional experience in the processing of cottonseed either for oil or protein. However, as biochemists and engineers we have immersed ourselves in the literature of this field and have picked the brains of many top specialists. If the position that represents the UNICEF consensus sounds conservative, you must realize that this reflects our conviction that several significant parts of the total problem do not appear to be adequately settled. While our agency must accept some calculated risks, it feels that it must also retain considerable caution in judging whether some nontraditional foods for children are indeed entirely safe and ready for unrestricted recommendation and promotion.

First on the list of problems is that the traditional industrial methods for processing cottonseed for oil, that is either by screwpressing alone or by prepressing and solvent extraction, usually yield a byproduct in which the nutritive value of the protein is invariably lower than that of the unprocessed seed. Many of you will recall that a major exercise of the first conference of this series held here three years ago was to define, as clearly as possible, the technical parameters for an edible cottonseed flour processed in this way. Cottonseed meats after decortication and prior to oil extraction are thoroughly cooked in order to improve the yield and color of oil. As is well known from extensive studies by a number of individuals, including many in this room, this improvement in oil processing efficiency penalizes the quality of the byproduct since it involves a Maillard-type of binding of gossypol and related pigments with the protein moiety. Unfortunately, the epsilon amino group of lysine, which is quite free in the unprocessed protein, is most sensitive to this type of binding. The result is, that whereas the quality of the oil and the efficiency of its extraction is improved, the nutritional quality of the protein is usually impared. Much effort has been expended to modify this process in order to safeguard the residual protein quality and yet retain reasonably efficient oil extraction. With milder and carefully controlled cooking, adequate amounts of unreacted lysine may survive to yield, as I have indicated before, a relatively nutritious product.

Next on the list of problems is that in order to produce a heat-processed product with acceptably low levels of free and total gossypol, sound cottonseed with not more than approximately 1.00% of total gossypol must be selected as a raw material. Excessive gossypol aggravates the risk and extent of lysine destruction. It is obviously difficult by conventional processing to provide uniform end products from a material as heterogeneous as cottonseed may be as grown in many countries. The requirement for soundness and low gossypol seriously limits the choice of seed often to an extent that most of the seed available in a given area, if not indeed in an entire country, would not qualify for edible processing.

Another aspect of the compromise is the precise and difficult control which must be exercised in the cooking and pressing operations in order to secure adequate efficiency of oil extraction, and yet retain a reasonably good protein nutritive value. Our experience indicates that the level of sophistication and experience required may not be readily available in countries or individual plants where such foods need to be processed.

An additional element of the compromise is the fact that while after processing the gossypol may be mostly in the bound form, it nevertheless remains in the product. The intense yellow-green color which it imparts to food mixtures in concentrations necessary for effective protein supplementation may have certain advantages which we are not aware of, but we are convinced that in most food applications, this is definitely a drawback to acceptability. We in UNICEF also continue to be uneasy about the apparently unsettled argument as to what a safe level of free gossypol is and even whether bound gossypol in appreciable concentrations in the diets of under-nourished infants and children is entirely safe when fed over considerable periods of time.

What I have been trying to say, in a nutshell, is that it seems to us that any modification of conventional screw-press or prepress solvent methods which requires appreciable heat processing imposes such stringent limitations on the quality of acceptable raw materials and demands such high levels of sophistication for processing and overall quality control, that we are dealing with a complex technology beyond that with which many oil mills can cope. Several speakers to follow me will doubtless disagree with these contentions. I sincerely hope that the evidence they present will be so overwhelming as to settle once and for all some of these questions which continue to disturb us.

The compromise I have tried to rationalize is further highlighted by the tantalizing promise which some new processing approaches for dealing with gossypol and related pigments seem to show. There will be a report later in this program of the status of technology being developed at this institution to degossypolize cottonseed by simultaneous extraction of oil and pigments. A process already operating commercially in Italy will also be described. The Texas Cottonseed Products Research Laboratory and perhaps others are also actively involved. Several months ago Dr. Fisher and his staff were kind enough to provide to UNICEF for infant feeding tests, a quantity of cottonseed flour produced from decorticated meats by the mixed solvent process. This material had been subjected to minimal heat treatment. We were immediately impressed by the light color of the product and its excellent nutritive value as indicated first by available lysine content, virtually equal to that in unprocessed seed, and subsequently confirmed by animal feeding trials. In fact the Protein Efficiency Ratio for this product was in the range of that to be expected in a properly heat-treated soybean oil meal. We had never before seen a processed cottonseed product with this nutritional quality. Quantities of this product for clinically supervised infant feeding tests were made available to Dr. Behar of INCAP in Guatemala and to Dr. George Graham at the Anglo-American Clinic in Lima, Peru. Dr. Graham has written recently to advise that in his metabolic studies with infants this product is definitely superior to the commercial heat-processed screw-pressed U. S. cottonseed flour.

We are fascinated also by the first protein quality data relating to glandless cottonseed varieties which will be discussed in considerable detail later in this meeting. We are pleased that the National Cottonseed Products Association is now strongly promoting the use of these new varieties. We hope that there will be constructive discussion at this conference as to what might be done to acquaint cottonseed growers in developing countries with the advantages of these new varieties in order to hasten their introduction and propagation in such countries.

On balance, it seems to us in the international agencies, that the solvent degossypolization techniques hold the greater immediate promise for a really effective break-through toward providing edible cottonseed flour as human food in a number of developing countries. The major promise of this technology, if perfected, is that it may require considerably less stringency in the acceptable gossypol levels in raw material, and hopefully also in view of minimal heating, greater latitude in processing control. We do not visualize that the immediate demands for such an edible product would be so great in developing countries as to justify plants with capacities used traditionally for oilseed processing. The ideal as we see it would be a food-grade operation providing about 10 tons of product per day, erected adjacent to a conventional oilseed plant and drawing from it the decorticated cottonseed meats as well as power, steam, etc. Presumably the oil refining stage of the process needs more study and development, but we hope that our guest from Italy will indicate that perhaps this problem too is near solution. I am personally convinced that the greatest opportunity for such products in tropical areas would be not necessarily as human food but as the protein component of animal feeds for monogastric species such as swine and poultry. This technology might well be the key therefore to a sharply expanded production of animal proteins in the tropics.

In a more philosophical vein I would ask that we speculate together on the question: How much technological experience or even empirical art must there be accumulated with any new processed food before it will be readily accepted and utilized on an effective scale by a human population? Clearly the information we have on cottonseed protein concentrates and ways to use them in food is very sparse. Consider for a moment the contrast here to the monumental technical literature relating to the properties, processing, and food uses of wheat and its products--or of malt beverages--or of dairy products. These materials were, of course, human food staples for thousands of years when only traditional or empirical processing methods existed. With the urgency existing in world food problems today, however, we simply cannot wait for such arts to evolve slowly and empirically in order eventually to rationally introduce oilseed proteins into human diets. As scientists concerned with food and its processing, we must accept the challenge of critical need for a tremendously broadened research attack on oilseed protein materials. We must explore new alternatives for their processing as human food. Intensive studies are needed on their properties, both chemical and physical, and on the way in which processing affects these properties. We need new or more refined methods for quality and sanitary supervision of their processing. We need to know much more about physical and chemical interactions of the constituents of oilseeds, and also their reaction with other components of balanced food mixtures when processed together. Our friends in India are asking insistently whether the use of conventional petroleum solvents is indeed as safe as we apparently believe it to be--and for which we have insufficient direct evidence. There is evidence from recent research that certain solvents used in food processing tend to destroy or somehow modify essential amino acids such as methionine, thus reducing their nutritive availability. We do not even know with certainty why cottonseed materials have recently been implicated as sources of toxicity to fish.

And finally, we must face the question: If all this unfinished business exists and if it is important, who has the responsibility to deal with it? Should U. S. scientists living in a country well able at this moment to tolerate the calorie and protein conversion losses required to sustain a surfeited animal protein economy assume responsibility for this problem when the benefits of any research they might do would apparently be to the advantage of countries other than their own? This is clearly a difficult but nevertheless challenging question for those who control the resources and motivation of U. S. scientists. In the international agencies we have recognized that the developing countries themselves must accept this responsibility and must train and equip their own scientists for this work. Unfortunately, this approach, as vital as it is and as strongly as we will encourage it, will not produce the required answers at an early time commensurate with the need. I return then to the challenge of these problems to science in all economically advanced countries. We are convinced that those who have helped to define these urgent problems do have a responsibility for their solution.

DISCUSSION

L. E. Allen: Thank you, Dr. Milner. Are there any questions on his paper?

L. H. Bean: This is a question of information merely. Dr. Milner says that within a couple of days we will have the report of the conference which was held last October in Rome. Some of us are interested in the results of that conference and will shape our remarks here from that report. I wonder if Dr. Milner could give us the gist of the outcome of that conference. I am not asking for the unnecessary, but it might be helpful to some of us to know what the importance of the conference was in the way of the conclusions or suggestions.

Max Milner: The conference referred to, the title of which was the FAO-UNICEF Conference with Food Industries on Protein Rich Foods, was held in Rome in the latter part of October 1963. Representatives were present from commercial firms, as well as of governmental and academic agencies, from about 19 countries. This was a working type conference. A series of briefing papers were presented covering the history of the effort in oilseed protein foods and other new proteins, but the major work was done in several committees dealing with the various technical and commercial aspects of the job. For example, as I recall, there were five committees; one dealt with processing; another with problems of quality control; a third dealt with marketing problems; a fourth with packaging; and the fifth with economic feasibility.

We had, I think, some of the best people one could find in the international food field who put their heads together and worked very seriously on these subjects for the full four days of the conference. The results of their efforts are put together in the report which has been referred to. I think that the nature of the report is almost predictable; namely that we have just embarked on this effort. We are still looking for answers. There is no question, however, as I tried to indicate, that a number of useful beginnings have been made. Several products are ready for exploitation, both commercially and for acceptability testing, and there is much work going forward.

But the most difficult part, of course, for commercial firms, is the question of producing low cost, protein-rich foods under circumstances where profits would be restricted for a long time. I am glad to say that some commercial firms have accepted this challenge with the idea that their investments will need a longer time to prove fruitful than they are traditionally used to.

These are generalities, Mr. Bean, but if anyone here would be interested in a copy and would like to have this information in more detail, he should leave his name with Dr. Kapsiotis, and we will have a copy sent to him. If any of you have any further questions, I will be glad to talk with you personally.

TRENDS IN THE COTTONSEED PROCESSING INDUSTRY IN THE UNITED STATES

by

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The supply of raw materials processed is one of the more important factors which stimulate changes in processing. Therefore, it would appear desirable in this discussion of trends in cottonseed processing to consider the important trends in U.S. production of cotton and cottonseed. Cottonseed is a valuable product of the production of cotton since its farm value is approximately 10% of the total value of the cotton crop at the farm level (1). However, it is a byproduct of the production of cotton lint and its volume depends upon the factors which determine the level of cotton lint production.

The 1947-61 trend in U. S. cotton production was slightly downward with an average yearly decline of 0.2% or 23,000 bales (2). However, production in 1962 and in 1963 was higher than the 1957-61 average (3). During the 1947-61 period, harvested acreage trended downward at the average annual rate of 4.4%, or 858,000 acres, and yields trended upward at a generally compensating rate of 4.5% or 16 pounds per acre per year (2). The most important cause of the sharp decrease in acreage was Federal government acreage allotments which have now reached the minimum permitted by present law. The upward trend in acreage yields may be generally attributed to (a) use of improved production practices, including irrigation, and (b) shifts from less productive land to more productive soils both within and between regions. It appears that growers have been able to compensate for the loss of acreage by increasing yields.

While production remained fairly static in U. S. totals, interesting contrasts developed between that of the four principal cotton producing regions (2). It trended upward at the average annual rate of 3.8% in the West and 0.9% in the Southwest during the 1947-61 period. On the other hand, it was slightly downward at the rate of 1.4% in the Delta and more sharply at 3.9% in the Southeast. In 1962 and 1963 there was some reversal of these trends between regions. Acreage yields increased in all regions, but there were sharp contrasts between regions in harvested acreage trends during the 1947-61 period. Western acreage remained about constant while the other three regions declined annually over a range of 4.0% to 6.5% less.

The annual production of 10 to 16.5 million bales of cotton lint during the postwar period has resulted in the production of 4.1 to 6.7 million tons of cottonseed annually (1). The 6.4 million tons of seed produced from the 1963 crop was the largest since 1953. During the 1936-45 period, approximately 81% of the farm production of cottonseed was processed by cotton oil mills. In the five-year period of 1946-50, approximately 88% of the production was crushed. The percentage of seed crushed increased to 91% during the period of 1957-61.

Alderks reported that in the 1945-46 crushing season approximately 95% of the cottonseed was processed by hydraulic press and that although several solvent extraction plants were under construction none was in successful commercial operation (4). During the postwar period there were rapid shifts in types of processing employed with cottonseed (5, 6, 7, 8). As is shown in Table I, the percentage of seed processed by hydraulic

TABLE I. --Percentage of cottonseed processed by various types of equipment

Type of Process	1945-46 %	1951-52 %	1953-54 %	1957-58 %	1962-63 %
Hydraulic Pressing	95	57	41	13	3
Screw Pressing	5	31	37	58	59
Solvent Extraction	0	12	22	29	38

pressing had declined from 95% in 1945-46 to 3% in 1962-63. Solvent extraction had gradually increased until it represents 38% of the volume in 1962-63. Screw pressing has shown the most increase, but most of these gains were achieved up to the 1957-58 crushing year. In the late 1940's it was believed by many that solvent extraction would expand very rapidly in the cottonseed crushing industry as was the case in the soybean processing industry. However, several factors contributed to more emphasis being given to conversion to screw pressing. Some technical difficulties were encountered in early cottonseed solvent extraction plants which appeared to indicate at that time that cottonseed was not well adapted to solvent extraction. While these difficulties were solved promptly, they interrupted the trend to solvent extraction. A very important factor which guided many decisions against conversion to solvent extraction was that of cost investment as weighed against available supply of raw material which could be foreseen. This was especially evident in some areas where cotton production was declining. Consumer acceptability of cottonseed meal also contributed significantly to many decisions to convert to screw pressing rather than solvent extraction. In contrast to the soybean industry which converted rapidly to solvent extraction, the cottonseed crushing industry had traditionally found the beef cattle and dairy producers to be the foundation of their meal markets. Some early difficulties had been experienced in pelleting solvent cottonseed meal and considerable pressure came from cattlemen to discount solvent meal prices. One of the principal reasons which support solvent extraction has been the higher oil yield. With the advent of greatly expanded soybean production vegetable oil prices were seriously depressed which further supported the trend to conversion to screw pressing rather than solvent extraction. In consideration of these factors, and others, many cotton oil mill operators appeared to agree with the following conclusion which was published by Brewster and Clark (9) in 1957:

> "When volume is sufficient to show a profit on new investment in conversion to both the screw-press and the prepress-solvent types of plant, the profit is always greater in converting to the screw-press type of operation."

However, as is apparent from Table I, some did not agree and solvent extraction has continued to make progress. Some of these latter decisions undoubtedly have been influenced by the availability of other oilseeds, such as soybeans and safflower, for which solvent extraction is highly desirable from a competitive standpoint. The demand for prepress solvent extracted cottonseed meals for use in non-ruminant rations may also have influenced some decisions to convert to solvent extraction.

The trend to conversion to screw-press or solvent plants, which in addition to being more efficient in extraction of oil, were generally of larger daily capacity, was accompanied by a trend to fewer mills (10). At the beginning of the postwar period approximately 350 mills were active in cottonseed processing. At the present there are approximately 180 mills which remain active in crushing of cottonseed. This is a loss of nearly 50% of the original number or about 10 each year. This trend in loss of numbers of mills does not represent the same loss in crushing capacity. A considerable excess of crushing capacity above available supply of cottonseed has existed, Beltwide, for many years. Also, average capacity per plant has increased as the number of mills decreased because of the higher capacity of the screw-press and solvent plants installed to replace hydraulic or older screw-presses. While the number of mills decreased by 109 from the 1947-48 crushing season to that of 1957-58, USDA reports indicated that the crushing capacity remained about constant (7, 11). The crushing of 5.7 million tons of seed in 1948-49 was reported to represent 60% of available capacity and the 4.2 million tons crushed in 1957-58 was 45% of mill capacity.

Throughout the modern history of the cottonseed crushing industry the yield of cottonseed oil, per ton of seed, has increased slightly through the more efficient operation of the equipment (11). As might be expected, the trend to screw-press and solvent extraction during the postwar period accelerated sharply this improved efficiency (1, 12). The unweighted average number of pounds of oil recovered per ton of seed from the 1946-49 crops was 317 pounds. That for the 4-year period beginning with the 1957 crop was 339 pounds. Peier and Gilliland (7) reported that for the 1957 crop seed, hydraulic pressing yielded 308 pounds of oil per ton, screw pressing yielded 327 pounds, and 376 pounds were obtained by solvent extraction. Since that time, the trend to use of high speed screw-presses has increased yields obtained in that process. Also, further increases in efficiency have occurred in direct solvent extraction.

At the beginning of the postwar period, approximately 900 pounds of cottonseed meal were produced per ton of seed. The average yield was 924 pounds for the 4-year period beginning with the 1958 crop of seed (1, 12).

Average figures for amounts of products produced per ton of seed can be very deceptive unless careful consideration is given to the individual specific factors which determine these at any given location. In addition to differences in processing equipment and methods of operating the equipment, there are tremendous differences in seed analysis for oil and nitrogen between years and between regions during any given year.

Generally, the protein content of the cottonseed meal produced trended downward in the prewar and war years and it became fairly well standardized at 41% even though a considerable amount of 36% protein meal has been produced in the older cotton areas such as the Southeast. With the advent of increased usage of cottonseed meal in poultry rations in the West, beginning in the late 1950's, there has been a trend toward production of 44% protein meal in that region. There has also been some interest in a 48-50% protein product there. Industry members generally appear to feel that such a higher protein product can be produced with present knowledge and equipment, and that it will be produced when and if price differentials exist which justify the change. It would be anticipated that the advent of glandless cottonseed on a commercial scale would provide further stimulation of higher protein values.

Product usage materially affects the processing conditions employed in the preparation of protein concentrates. Therefore, it is of interest to note the manner in which cottonseed meal has been used. Traditionally, the market outlet for cottonseed meal has been as a feed for the ruminant animals--beef cattle, dairy cows, and sheep. For the year beginning in October 1947, USDA estimates (13) the percentages of cottonseed meal consumed by the various classes of livestock and poultry were: beef cattle, 43.0%; dairy cattle, 32.9%; sheep, 3.1%; hogs, 10.2%; and poultry, 5.1%. These estimates indicate that in 1961 there had been very little change in beef cattle and sheep consumption (45.6% and 2.0% respectively); dairy cattle consumption had decreased almost one-third to 23.6%; swine consumption was even less than in 1947 at 6.0%; and poultry consumption had materially increased to 18.0% of the total or 353% of the 1947 level of poultry usage. This increased poultry consumption which provides an additional market outlet for 350,000 tons has occurred primarily on the West Coast where degossypolized cottonseed meals of low residual oil content are used in amounts up to 10% of rations fed laying hens.

Published data (1, 12, 14, 15, 16) show an increased usage of cottonseed meal by feed manufacturers during the postwar period, but the trend is much less intense than would be expected in view of the increased production of mixed feeds and the known increase in use of cottonseed meal in poultry feeds. Twenty-one percent of the production of cottonseed meal was used in manufactured feeds in 1947. This percentage had increased to 27% in 1954 and was only a fraction of a percentage higher in 1961. It should be noted that Decossas et al., estimated in 1962 that 1.1 million tons of cottonseed meal was used by feed manufacturers during the 1960-61 season (17). This would be about 45% of the total amount of meal used for feed.

Although nutritionists generally agree that cottonseed meals with low free and total gossypol and high nitrogen solubility values are desirable for use in rations fed nonruminants, complete agreement is lacking upon the optimum levels of these for such usage of the product. Nevertheless, they are sufficiently well accepted to justify a brief look at the trends in nitrogen solubility and free and total gossypol levels. For this purpose we have reviewed the analyses of several groups of cottonseed meals which were collected as being representative of production in 1953, 1963, and intervening years (18, 19, 20, 21, 22). These data show a wide variation in the three analytical values between the methods of processing, i.e., screw pressing, prepress solvent, and direct solvent, and between meals produced by the same process. However, the data do not indicate that there has been a trend toward higher or lower values for nitrogen solubility, free gossypol, or total gossypol during the decade covered by these analyses when each process is considered separately. For nitrogen solubility, direct solvent meals ranged from 69% to 83%; prepress meals were 69% to 83%; and screw-press meals were 28% to 68%. Direct solvent meals were highest in free gossypol with values between 0.22% and 0.40%. Prepress solvent meals ranged from 0.014% to 0.09% and screw-press meals contained 0.02% to 0.09% free gossypol. The highest values for total gossypol were reported for prepress solvent meals at 1.64% and with a downward range to 0.66%. The direct solvent meals contained from 1.00% to 1.08% total gossypol and the screw press meals ranged from 0.57% to 1.39%.

These analytical data tend to support the conclusion that some of the processing equipment changes which have occurred in the postwar years have made available cottonseed meals which are better adapted to use in nonruminant rations. It appears that this is true largely because the prepress solvent extraction system reduces free gossypol and residual oil to low levels without the need for extreme processing heat. The extreme variations in analytical values which occur between meals of all major processing methods strongly suggests that quality control in seed and processing conditions can effect improvement in nutritive quality for nonruminants. This indication is well supported by the experience of some individual mills which have been able to produce a uniformly high quality meal, as judged by these analytical criteria and by biological tests. Nevertheless, the lack of an apparent clearcut trend toward analytical values which would indicate less heat damage during processing suggests that, industry wide, mill operators have not been convinced that it is profitable for them to make these refinements in their processing techniques.

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DISCUSSION

L. E. Allen: I wonder, Garlon, if you could give us a quick summary of the NCPA projects on education and research just as a matter of general interest for us today.

G. A. Harper: Briefly, the research program of the Association relates to efforts to solve quality problems which may limit the greatest degree of usefulness of cottonseed products. We are searching for the most practical solutions and we are interested in the earliest possible application of results. One of the major lines of work is that of developing and testing glandless cottonseed to eliminate the gossypol problem in all of its ramifications. It would be inappropriate to devote time here to this discussion because two papers will be presented on the subject later in the conference. Another major approach is the attempt to develop cottonseed resistant to deterioration in the field and in storage because of decreased permeability of the seed coat or hull. Results up to this time have been promising, but it has not been established that practical application can be achieved.

The Association has not provided support for the mixed solvent extraction process developed by the Southern Laboratory, but we have been most interested in it and we are anxiously awaiting developments which will determine whether it can become a commercially acceptable process. Another major area of interest is that of cottonseed lipid fractions which contribute to the production of pink discoloration of egg whites. We have been pleased to have the opportunity to support fundamental studies of the structure of the cottonseed in Dr. Altschul's Pioneering Research Laboratory. Many other lines of investigation are being supported or encouraged by the Association. Time does not permit a detailed discussion of them at this point.

G. S. Sidhu: Would you kindly say if there is a good commercial possibility in the future of dehulling first and removing the lint later on, because this process would naturally yield a cottonseed flour with higher protein content and better nutritive value?

G. A. Harper: That question was, could you separate the hulls first before separating the lint in processing? This is certainly quite a departure from the process which is used at the present time; I cannot answer this question. There may be people in the Conference who are interested in this and might have done some work on it. If so, I would be very glad to defer this question to them. Ralph Hutchins, would you have any comment in this regard?

Ralph Hutchins: My comment is the same as yours, I don't know.

G. A. Harper: Cecil Wamble, do you want to comment on this question?

A. C. Wamble: It can be done. It depends a lot, you know, on how much lint is left on the seed after it is ginned. A good clean ginned seed could be separated with a minimum of separation losses. A better solution is to take off a light cut of lint which usually is valuable enough to support the cost of removing it. It doesn't take a lot of equipment. I would highly recommend if one were going to attempt to vary the process much that he take off some lint, about ten or fifteen pounds. With a unit like Bauer Brothers' #153 you can do a good job of separation. You would probably have to run the hulls through a second huller or over a shaker in order to recover meats from a few uncut seeds.

A. M. Altschul: Might I comment on that. If I understand your question, Dr. Sidhu, the point would be that you would have less of the hulls in the final product. But then, of course, there are alternatives to removing the lint first and that is to have better cleaning equipment either before or after removal of oil to remove most of the residual hulls.

I wonder if there are people in the audience who could talk about how low you could get in residual hull content by proper cleaning equipment. Charlie Hay, do you want to say anything about that?

Charlie Hay: I think probably Cecil could give you more information on that than I could.

A. M. Altschul: Cecil, would you like to comment on that?

A. C. Wamble: You could get very low. Allen up there has some figures on Proflo, which is a human food product being produced now, and he knows about what the crude fiber is running on that material. Those people are doing that every day, have been doing it for years. Layton, what are some of those values?

L. E. Allen: Cecil, I would have to defer this to Gene Cochran, who is representing Traders Oil Mill at this Conference. If he would wish to speak about it, I would be very happy to have him do so.

G. B. Cochran: The fiber content is usually less than 3% in Proflo. We normally bring down the hull content to less than 1%.

C. M. Lyman: It seems to me that the reason for the question is to get all the hulls out for the production of cottonseed flour for human use. Certainly this can be done and the production of Proflo demonstrates that it is possible, however, we all know that the equipment required is rather extensive. I merely rose to point out the alternative of separating a small fraction of whole meats in a mill designed to produce animal feed. By this procedure the finely divided hulls are left in the animal feed. This requires so much less equipment that I think it is worthy of consideration.

L. E. Allen: That is certainly one way of doing it. I did not want the Conference to get involved at this time in the technicalities of cottonseed processing because we have many speakers who will discuss different phases of it within the forthcoming sessions.

Are there other comments or questions particularly pertaining to Garlon Harper's presentation on the trends in the industry? If not, we will move on.

DOMESTIC AND FOREIGN MARKET POTENTIALS FOR FOOD PROTEIN PRODUCTS

by
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Introduction

In discussing the world-wide market potentials for high-protein concentrates, the presentation will be divided into two parts.

First, I will summarize briefly the magnitude of the world food deficit as projected by USDA in the World Food Budget. Then, the changing nature of world food needs will be discussed with specific reference to: (1) the broad parameters affecting world food availabilities, commercial imports, and food aid programs as they relate to the nutritional gap; (2) the changes that are expected in future food balances for highly developed, moderately to rapidly developing, and slowly developing countries; and (3) the inadequacy of world food distribution systems.

Wayne Bitting will then discuss some of the economic considerations relating to the efficiency of nutrient sources, with particular reference to plant and animal proteins. After Wayne's comments, I will attempt to point out the significance of economic factors affecting costs, types, and qualities of protein concentrates as they relate to the development of market outlets in groups of countries experiencing different rates of economic development.

Magnitude of World Food Deficits

In recent years much effort has been devoted to the analysis of world food needs and ways of reducing the gaps between needs, consumption levels, and production. The U. S. Department of Agriculture[1] and international groups, particularly the Food and Agriculture Organization of the United Nations, and Organization for Economic and Cooperation Development,[2] have been active in this field. Another group concerned primarily with the nutritional aspects of world food needs is the Interdepartmental committee on Nutrition for National Defense of the U. S. Government. This Committee has been active in conducting nutritional surveys in selected countries for the purpose of identifying nutritional inadequacies and to assist in efforts to improve nutritional levels.

The concensus of these studies is that world food needs exceed current as well as anticipated food availabilities. This situation has persisted for many years and will likely continue into the future.

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[1] In the U. S. Department of Agriculture, the Economic Research Service's Regional Analysis Division's research has been concerned with the development of World Food Budget estimates, which project world food balances on a country to country basis with summaries on a regional and world basis. The results of the first world food survey were first published in 1960 under the title of "Food Balances in Foreign Countries". In March 1961 "The World Food Deficit--A First Approximation" presented regional estimates of world food deficits for 1958 with projections for 1962 and 1965. This was followed by a more comprehensive analysis, country by country which was published in October 1961 under the title, "The World Food Budget, 1962 and 1966". The world food budget is in the process of revision, based upon new food balances for 1959-61.

[2] The results of FAO's research and analysis on world food balances were published in 1962 under the title of "FAO Commodity Projections for 1970." The OECD has not published a detailed country by country or regional compilation of world food balances, but certain of its functioning committees have devoted considerable time to the review and appraisal of world food needs and means of meeting them more effectively in the future.

There are several ways in which world food balances or nutritional inadequacies may be estimated. Lester R. Brown in his study, "Man, Land, and Food" lists three major approaches for estimating world food deficits as follows:[3] (1) the empirical approach employed by economists based on broad aggregations of data on food production, trade, and availability, (2) the scientific approach employed by doctors, physiologists, and nutritionists involving detailed physical examinations of limited numbers of persons for the purpose of identifying the symptoms of malnutrition, and (3) the national dietary or household surveys that are made in selected countries where aggregate data is incomplete. The World Food Budget projections are based primarily on the empirical approach, but as the results of the national food or household surveys become available, they are used extensively in the refinement of the budget estimates.

The aggregate world food shortages as estimated in the World Food Budget projections for 1966 were broken down into four major food groups to indicate the relative magnitude of nutritional deficiencies as follows:[4]

Animal protein, in terms of nonfat milk solids	1.5 million metric tons
Pulse protein, in terms of dry beans and peas	0.2 million metric tons
Other protein and calories, in terms of wheat	29.3 million metric tons
Fat in terms of vegetable oil	1.9 million metric tons

These shortages represent the gap between actual amounts of food that people in deficit countries have available, whether domestically produced or imported, and the larger amounts of food needed to maintain normal physical activity and health as determined by international reference standards.[5]

Value of World Food Deficit

The physical magnitude of the world food deficit may be expressed in monetary terms as shown in Figure 1. As indicated in Table I, the world food deficit projected for 1966 is the equivalent of approximately $3.4 billion. This is nearly double the present U.S. food aid program authority of $1.8 billion annually under Titles I and II of the P. L. 480 program.

On the basis of these projections animal proteins on a value basis represent approximately 11 percent of the world deficit or

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[3] Brown, Lester, R., "Man, Land, and Food - Looking Ahead at World Food Needs", U.S. Department of Agriculture, Economic Research Service, Regional Analysis Division, November 1963, Foreign Agricultural Economic Report No. 11, p. 35.

[4] The World Food Budget--1962 and 1966, Economic Research Service, U.S. Department of Agriculture, October 1961.

[5] In the calculation of the projected nutritional gap for 1966, the following criteria were used in establishing minimum nutritional levels.

The reference standard for animal protein was established at 7 grams per person per day or 12 percent of the total protein. The reference standard for pulse protein was the amount of protein that must be added to the available animal protein to bring the total up to 17 grams. The reference standard for total protein was established at 60 grams per person per day. The residual deficit between 17 and 60 grams or approximately 71 percent of the total protein deficit is expressed in terms of the amount of wheat required to bring the total protein level up to 60.

In selecting wheat as the basis for quantifying residual nutrient needs it should be pointed out that wheat is of considerably higher protein content than the other principal food grains--rice, corn, sorghums, etc. Since lower protein content grains account for a rather high proportion of the national diets in many areas of the world such as the Far East. Africa and Latin America, it may be concluded that the actual protein shortage that must be made up from sources other than grains as quantified in the budget projection is understated. From a practical standpoint, then, it is apparent that there is a great need for sufficient or low cost protein concentrates to meet nutritional needs since there is not enough wheat to fill the deficit as projected in the World Food Budget.

In addition to the wheat required to meet the minimum protein reference standard as calculated in the budget projections, some additional wheat or the equivalent of other grains would be needed to bring the calorie standard up to 2,300 for the Far East and ranging up to a high of 2,710 for countries such as Canada and Russia.

The minimum reference standard for fats used for calculation of projected needs was established as the amount necessary to provide 15 percent of the minimum standard calorie level.

Table I. -- Calculated value of world dietary deficit, by food nutrient group and by geographic region, projected for 1966[1]

Food nutrient group and geographic region	1966	Percent of total
	Mil. dol.	Percent
Animal protein[2]		
Far East	162	4. 8
Communist Asia . .	183	5. 5
West Asia.	0	0
Latin America . . .	0	0
Africa	15	0. 4
Total	360	10. 7
Pulse protein[3]		
Far East	16	0. 4
Communist Asia . .	0	0
West Asia	0	0
Latin America . . .	0	0
Africa	13	0. 4
Total	29	0. 8
Other protein & calories[4]		
Far East	1, 251	37. 3
Communist Asia . .	206	6. 2
West Asia.	82	2. 4
Latin America . . .	169	5. 0
Africa	150	4. 5
Total	1, 858	55. 4
Fats[5]		
Far East	446	13. 3
Communist Asia . .	639	19. 0
West Asia.	5	0. 2
Latin America . . .	13	0. 4
Africa	7	0. 2
Total	1, 110	33. 1
Grand Total	3, 357	100. 0

$360 million.6/ The projected shortage of pulse protein is fairly small. There is a real problem in assigning a value to the quantity of wheat required to meet protein needs above those included in animal and pulse proteins because wheat is a major source of food energy or calories as well as protein. National averages, of course, do not reveal, or in fact they may actually cover up important deficits for important segments of the population within a given country. On the other hand it is obvious that the nutrient balances of the diets for some segments of a country's population

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[1] Calculated on basis of the world nutritional deficits as compiled, from "The World Food Budget-1962 and 1966," ERS, U. S. Dept. Agr., January 1962. The value of nutritional deficits as presented in this table were calculated on the basis of average U. S. export prices for fiscal year 1960-61. Quantities were determined by subtracting average food (nutritional) availabilities per capita from average minimum food (nutritional) requirements as determined by international nutritional reference standards as published by the Food and Agriculture Organization of the United Nations in the "FAO Second World Survey, 1962."

[2] In terms of nonfat dry milk.

[3] In terms of beans and peas.

[4] In terms of wheat.

[5] In terms of vegetable oils.

[6] There is a factor that contributes to an understatement of the relative magnitude of the animal protein component. Value calculations were based on U. S. export prices. The export market value for nonfat dry milk for this calculation was slightly over 10 cents per pound, as compared to commercial wholesale unsubsidized prices of 16 to 18 cents per pound. Since the animal protein component was quantified in terms of the export market value of nonfat dry milk, it appears that the commercial value of the animal protein deficit might be nearer $650 million annually or nearer 20 percent of the dollar value of the total food deficit.

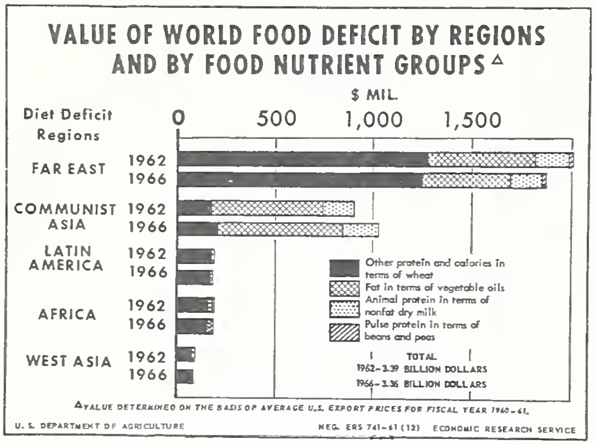

Figure 1

may be well in excess of the minimum nutritional reference standards used as a basis for the computations.[7] Therefore, it is not likely that food deficits would be eliminated if the world production of proteins, fats, and calories increased by an amount equal to the deficits as quantified in the World Food Budget projections. The projected deficits do not take into account the ability of countries to fill their food shortages. Ironically, those countries with the largest deficits are in the least likely position to meet food needs because of underdevelopment which contributes to such factors as low personal incomes, poor income distribution, inadequacy of marketing systems, including transportation and shortages of foreign exchange, and national export and import trade policies.

The Changing Nature of World Food Needs

Before delving into the market potentials for specific types of U. S. foods and food products and their relationship to world food needs, it may be helpful to review the nature of the broad parameters of world food needs and the ways in which they may be supplied. Such a review should be helpful to all research groups concerned with meeting food needs or reducing identifiable nutritional deficits. The parameters affecting world food production, consumption levels, and food availability vary greatly from one country to another depending upon the stage of development as well as the rate of economic development and growth. This makes it impossible to generalize. In this connection it may be helpful to look at three charts to illustrate schematically three different situations as follows:

(1) Food needs and consumption parameters at a given time as one moves from "rich" countries to "poor" countries.[8]

(2) Food needs and consumption trends over time for moderately to rapidly developing countries[9] and

(3) Food needs and consumption trends over time for slowly developing countries.[10]

These charts portray the relationships between dollar markets and food needs now and in the future for developed, rapidly developing, and slowly developing countries.[11] It is important to keep in mind that the stage and rate of development over time will influence requirements, needs and the absorptive capacity of individual countries for commercial imports as well as food aid. The illustrations in Figures 2, 3, and 4 are intended to emphasize some of the major variables and to indicate some of the hidden assumptions and hypotheses that are not too frequently brought into proper perspective.

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[7] The availability of proteins is probably the best measure of the quality of diets. Of course, quantity as well as quality must be considered. In reference to protein quality, it is assumed too frequently that vegetable proteins denote "poor" quality and animal proteins represent "high" quality. It may well be that this connotation results from the fact that vegetable proteins are relatively "cheap" in relation to the more expensive or costly animal proteins that are taken for granted in the highly developed or high income countries. In our discussions with the nutritionists we understand that cereal and plant proteins may be substituted for animal proteins and vice versa without any major effect on "quality" so long as the proper amino acid balance is maintained. The inclusion of animal proteins helps to achieve this balance but it does not necessarily follow that a high proportion of such proteins is essential to achieve a desirable balance. If it did we would have to conclude that the majority of the population of India and many other parts of the world with limited agricultural resource capacity would never achieve a desirable dietary protein balance.

[8] The connotation "rich" and "poor" in this illustration as related to nations implies a range from the high per capita purchasing power of the equivalent of $350 or more annually as found in the developed or industrialized countries to the lower per capita purchasing power of less than $350 annually in the underdeveloped countries. Countries in the high-income group are: United States, Canada, Australia, New Zealand, South Africa, and most of Europe including USSR, while those in the lower income groups are listed in footnotes 9 and 10.

[9] Countries falling in the moderately to rapidly developing group include: Algeria, Argentina, Chile, China (Taiwan), Costa Rica, Dominican Republic, Ghana, Iraq, Israel, Japan, Jordan, Kenya, Lebanon, Mexico, Nicaragua, Nigeria, Panama, Rhodesia, and Nyasaland, South Africa, Tanganyika, Uruguay, Venezuela, and Vietnam (So.). (Average rate of growth of per capita incomes over 1.5 percent annually.)

[10] Countries falling in the slowly developing group include: Afghanistan, Angola, Belgium Congo, Cambodia, Cameroun, Ceylon, El Salvador, Ethiopia, French Equatorial Africa, French West Africa, Guinea, Haiti, Indonesia, Iran, Liberia, Laos, Libya, Malaya, Morocco, Pakistan, South Korea, Togo, Tunisia, and Uganda. (Average rate of growth per capita income less than 1.5 percent annually.)

World Food Needs and Consumption at a Given Time "Rich" to "Poor" Countries[12]

Figure 2 shows the differences of the food supply components at a given time as one goes from "rich" to "poor" countries.

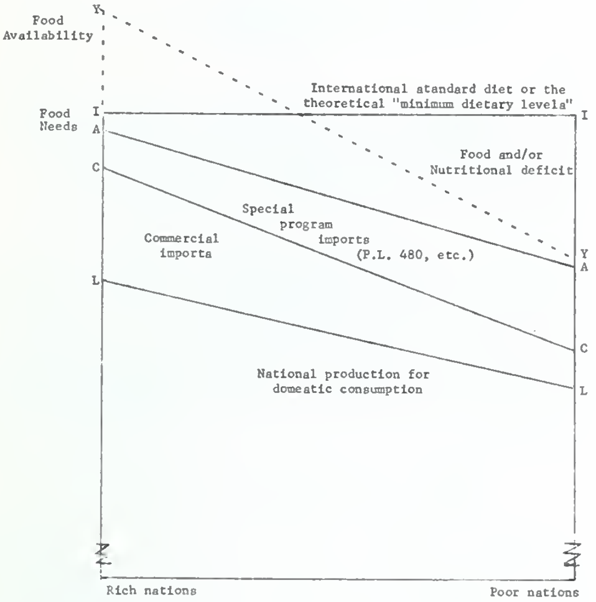
Y
Food Availability
International standard diet or the theoretical "minimum dietary levels"
Food Needs
I
A
C
L
I
Food and/or Nutritional deficit
Special program imports (P.L. 480, etc.)
Commercial imports
Y
A
C
L
National production for domestic consumption
Rich nations
Poor nations

Figure 2. -World food needs, availabilities, and deficits at a given time--rich and poor countries

The total rectangle in Figure 2 suggests the theoretical total or per capita amount of food needed to provide a predetermined "ideal" or calculated reasonable minimum nutritionally standard diet.[13] The dotted line YY extending above the rectangle is drawn into the diagram to indicate that the actual consumption of food in the rich countries may be far in excess of that required to meet minimum international reference standards. The bulk of the food available for domestic consumption by any country regardless of its stage of development or per capita income level is derived from national production. Note that the domestic production declines in volume in relation to national food needs as one moves from "rich" to "poor" countries. Commercial imports provide supplies in addition to national production. U. S. agricultural exports supply a substantial part of world commercial imports.

In recent years, P. L. 480 has helped many of the underdeveloped or "poor" countries more fully meet their food needs through concessional shipments, with the volume shipped to poor countries increasing in relative importance. The United States is the major supplier of concessional or special program imports. Despite P. L. 480 and other food assistance programs, there is still an unfilled gap between the level of consumption, line AA, actually attained in the poorer countries and the "optimum dietary level", represented by the II line.

It should be pointed out that wide disparities in per capita incomes within a country prevent many people in the high-income countries from obtaining the optimum dietary level even though average food consumption would indicate otherwise. The highly developed countries are the principal exporters of food, but many of the less developed countries also are important exporters of certain kinds and types of food even though actual food or nutritional deficits exist within the country.[14]

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[11] The grouping of countries as to developed, rapidly developing and slowly developing countries is based upon the grouping criteria utilized by Willard W. Cochrane, Arthur B. Mackie and Grover Chappell in their paper, "Potential Uses of Farm Products as Aid to Developing Countries," which was presented before the annual meeting of the American Farm Economics Association, August 1963, Minneapolis, Minn., particularly tables 2 and 3.

[12] None of the lines on this chart are based on actual quantitative data for any particular country or group of countries. They are drawn for the purpose of illustrating relative overall trends and probable relationships. It would be purely accidental if the relationships were linear--rather the individual lines in reality bob up and down. In general the various lines represent the direction of expected changes.

[13] This chart does not take account of food losses resulting from spoilage, pests, etc., either for that produced domestically or imported.

The animal derived content of national average diets usually decreases, and the cereal content as well as the starchy roots and tubers content usually increases as one moves from "rich" to "poor" nations. As a consequence the poorer or underdeveloped countries as a group experience significant protein deficiencies.[15]

The level of food consumption (line AA) might be raised by making fuller use of seasonal or annual food production by reducing food losses. Such factors as waste, prices of food in relation to other prices, and development of a marketing and distribution system which would facilitate the efficient movement of local foods into consumption should be considered. Food losses resulting from spoilage and pests are relatively greater in the underdeveloped countries than in the industrial ones.

A major accomplishment of the P. L. 480 program has been to raise food consumption (line AA) above the level which the importing nation would have been able to support (line CC) in the absence of the program. Figure 2 suggests the need for several lines of research (nutritional, biochemical, and economic) to identify the kinds and types of food needed, to quantify with greater precision specific commercial and concessional export market potentials of U. S. farm products, and to determine the most efficient and practical means of meeting them.

Food Needs and Consumption Over Time

In projecting food needs and consumption trends over time it is apparent that present supply-demand relationships between the "rich" and the "poor" nations will be changing. Although it may not be possible to project the actual change over a specific period of time, it should be possible to indicate with considerable precision the changing direction of food balances between groups of countries developing at different rates through time.

Moderately to Rapidly Developing Countries:

In the case of the moderately to rapidly developing countries, opportunities for expanding domestic food production and consumption are much more favorable than they are in the poor or slowly developing countries. Figure 3 suggests that the moderately to rapidly developing countries are likely to (1) expand food production, (2) increase per capita consumption, (3) reduce food and nutritional deficits, (4) increase commercial imports, and (5) reduce or eliminate dependence on food aid shipments.[16]

Slowly Developing Countries:

The pattern for poor countries is less encouraging because of the large prospective increases in population and the slower rates of economic growth. Figure 4 suggests that the nutritional gap between actual

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[14] Factors contributing to dietary deficits in the less developed countries either in terms of actual quantities or in terms of poor nutritional balances may be due to such factors as: (1) individual domestic purchasing power deficiencies; (2) distribution obstacles; (3) deliberate choice of nonfood items over food; (4) food habits or preferences; or (5) a nation's stage of economic development which limits its ability to allot foreign exchange to import enough food to fill the deficit.

[15] Low individual purchasing power as well as poor income distribution, together with a country's inability to produce enough food to meet domestic needs or to allocate enough foreign exchange to import sufficient quantities of the kinds and types of food to supplement national diets, presents a formidable problem. Under such circumstances the relative costs of nutrient sources, particularly proteins take on major importance in the development of programs to reduce the world's nutritional deficit or to improve the quality of diets.

[16] The nutritional deficit in these countries is not likely to be eliminated for many years. As the earning power of these countries improves, increased allocations of foreign exchange to import food will make them more important as commercial markets. Nutritional needs and particularly the opportunity for improving the quality of diets will be large in relation to the financial ability of the rapidly developing countries to meet them fully. Consequently, the relative costs of foods supplying needed nutrients will be important, particularly sources of protein which are of primary importance in improving the quality of diets.

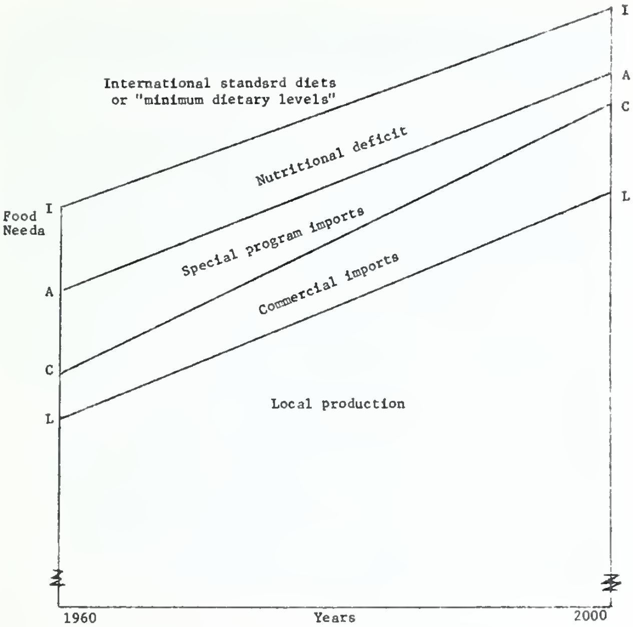

Figure 3.--Changing food needs, availabilities, and deficits for the moderately to rapidly developing countries through time

per capita food consumption and food needs as expressed by minimum nutritional dietary standards is not only larger in these countries than in the rapidly developing countries at the present time, but it appears to be widening over time.

There is heavy reliance on grains, tubers, and root crops in the poor or slowly developing countries. The need for improving the quality composition of diets is even greater than in the rapidly developing countries. Consequently, emphasis on the low-cost vegetable protein sources to meet nutritional needs as adequately as possible will be extremely important in these countries.

Implications of an Expanding Population

Two additional charts illustrate the dimensions of future world food needs.

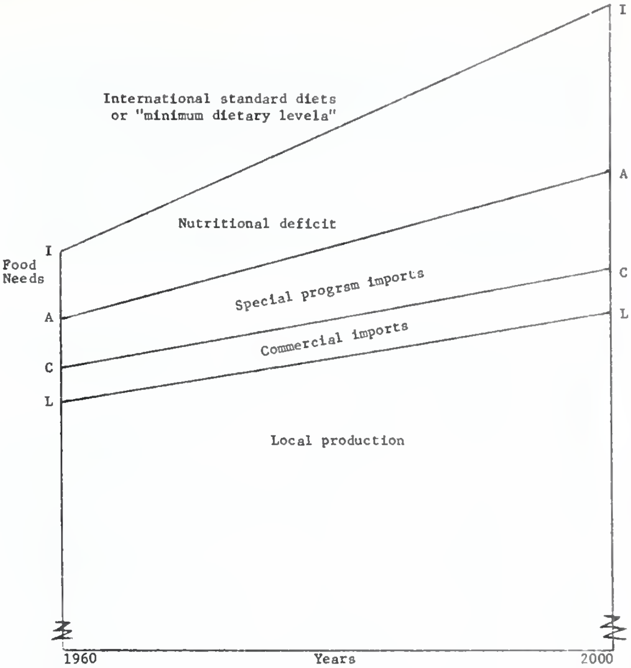

Figure 4.--Changing food needs, availabilities, and deficits for the slowly developing countries through time

Figure 5 shows the growing world food

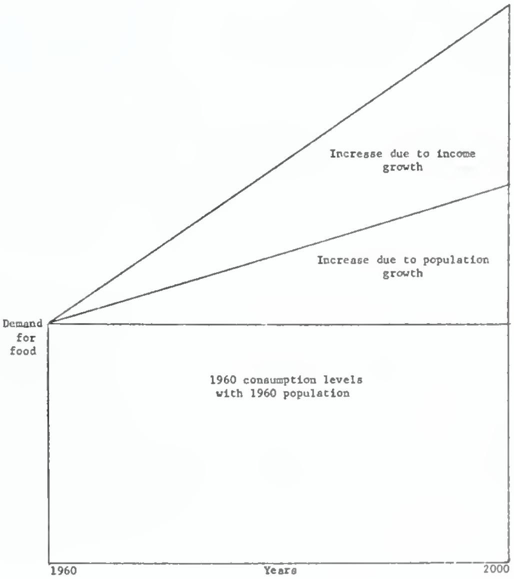

Figure 5.--Projected world food demand

demand--first, to meet the needs of an expanding world population, and second, to provide for dietary improvements accompanying higher per capita incomes.[17]

In illustrating the nature of increasing world food needs W. W. Cochrane commented as follows:

..."Generally, however, the nature of both the demand and supply of food can be meaningfully related to the stage of economic growth. For example, in countries well below the take-off stage in economic development, growth in per capita incomes and agricultural production is very slow. Increases in total demand for food are primarily a function of population growth. But, since population growth is often rapid, food requirements increase faster than food supplies. Under such conditions, the nature of the food deficit in these countries is primarily nutritional... countries experiencing a moderate to fast rate of growth in per capita incomes and agricultural production may have a food deficit problem that is both nutritional and economic. It is nutritional if the available supply of calories and proteins is inadequate when related to a minimum nutritional standard. It is economic when rapidly rising income per capita increases the total demand for food more rapidly than the supply of food. And ironically, once the take-off stage is passed, the economic food deficit has a tendency to widen with rapid and sustained economic growth."...[18]

To demonstrate the relative importance of increasing population and increasing personal incomes on increased food consumption, let us take a country such as Egypt with a high income elasticity of demand for all food where (1) population is increasing at an annual rate of 3 percent, (2) food production is increasing at an annual rate of 1 percent, and (3) the gross national product is increasing at an annual rate of 2-2/3 percent.

An equation for estimating future food consumption given by Kazushi Ohkawa states that the rate of increase in the consumption of food is the sum of population growth plus the product of the rate of per capita income growth and the income elasticity of the demand for food ($d=p+gn$). Using this equation a projection of the increase in demand for food can be made. Here d refers to the rate of increase in demand for food measured in constant monetary units; p is the annual rate of change in population; g is the annual rate of growth in per capita gross national product; and n is the income elasticity of demand for food:

$$d = p + gn$$

Using the equation with the following values:

$$p = 3$$
$$g = 2.67$$
$$n = .7$$

then $d = p(3) + g(2.67) \times n(.7)$

$d = 4.87$, or nearly 5 percent

If the past rate of increase in agricultural production--almost 1 percent per year--continues, this country would on the basis of these projections face an annual increase in demand for food of about 4 percent to be met from food resources outside of the country.[19]

Developed countries with a much smaller population increase, a much lower income elasticity for the demand for food, and a fairly high rate of growth would experience a much smaller increase in the consumption of food.

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[17] This chart is not drawn to scale and is used only to illustrate two of the most important basic factors contributing to the increasing future world need for food. The world demand for food at the 1960 level is somewhat below world food needs as determined by international reference standards in many of the developing countries.

[18] W. W. Cochrane, pages 4 and 5 of work cited in footnote 11.

[19] Based on an unpublished manuscript by Haven D. Umstott, "P.L. 480 and Other Assistance to United Arab Republic--Egypt", Development and Trade Analysis Division, Economic Research Service, USDA.

Figure 6 illustrates the rapid increase in world population anticipated over the next 20 to 40 years. Population estimates for 1980 are those used by Cochrane which projected the annual rate of population increase that occurred over the period 1953-60.[20] If the same rate of increase is projected to the year 2000, the world population will exceed 6 billion as compared to the 3 billion of 1960 (Table II).

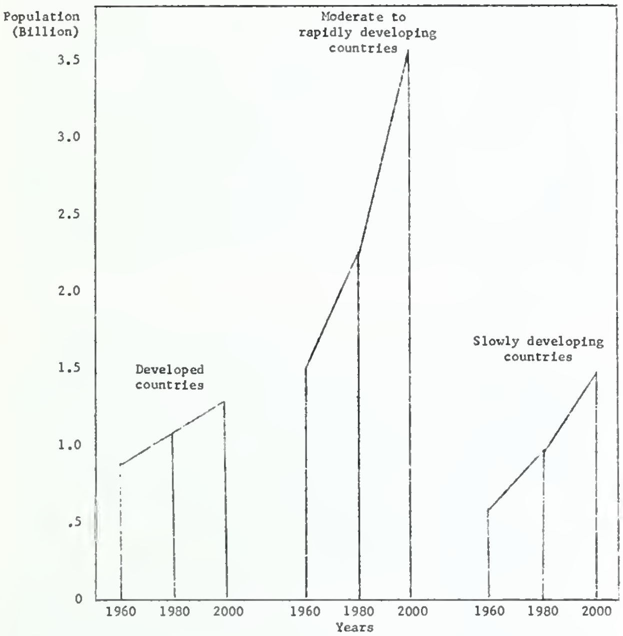

Figure 6. --World population projections developed, moderate to rapidly developing, and slowly developing countries, 1960, 1980, and 2000[1]

[1] The grouping of countries as to developed, rapidly developing, and slowly developing countries is based upon the grouping, criteria utilized by Willard W. Cochrane et al. in his paper, "Potential Uses of Farm Products as Aid to Developing Countries," which was presented before the annual meeting of the American Farm Economics Association, August 1963, Minneapolis, Minn. Particularly tables 2 & 3.

Without arguing the veracity of the straight-lined projections to estimate world population to the year 2000, figure 6 showing projections for the developed, rapidly developing, and slowly developing countries clearly illustrates the increasing magnitude of future world food needs.

Table II. --World population projections from 1960 to 1980 and the year 2000[1*]

Regions by rate of growth	Population estimates (billions) 1960	1980	2000
Developed	.9	1.1	1.3
Moderate to rapidly developing	1.5	2.3	3.6
Slowly developing	.6	1.0	1.5
World total	3.0	4.4	6.4

In terms of food needs due to population increases along, it is clear that the major food problem will be in the developing countries. In addition to the more rapid increase in population, per capita incomes are also expected to increase more rapidly--thus, increasing the economic needs for food that are associated with economic development.

Relationship of Personal Income and Quality of Diets.[21]

It has been known for a long time that both the level and pattern of food consumption are influenced by the level of income among countries as well as its distribution within a given country. For example, as income increases, the level of food consumption increases, particularly in the lower income groups; the pattern of food consumption shifts toward the more expensive foods, and the share of income spent for food declines.

The available supply of food energy in the industrial or developed countries with

[20] W. W. Cochrane, op. cit., table 2

[21] Much of this discussion on the relationship of income to the quality of diets is based upon Lester R. Brown's analysis which is presented in his publication, Man, Land, and Food, op. cit., pages 41-47.

[1*] Estimates based on projections of annual population growth for the period 1953-60. Developed countries, .1 percent; moderate to rapidly developing countries, 2.2 percent; slowly developing countries, 2.4. percent.

annual per capita incomes in excess of $600 averages upward from 2,900 calories per person per day as contrasted to per capita calorie availability of less than 2,400 in the less developed countries with annual per capita incomes less than $300. The calorie intake rises very rapidly as incomes increase in the lower income ranges, but as incomes move above $300 annually, the rate of increase of consumption slows down. After per capita incomes reach $1,000 per year, food energy consumption changes little with further increases in income.

The share of calories from starchy foods declines rather steadily as incomes rise, dropping from two-thirds or more in low-income regions such as Asia or Africa to one-fourth or less in high-income regions such as North America or Oceania.[22]

The level of protein consumption is quite responsive to changes in income. As in the case of energy foods, the response is greatest with increases in per capita income up to $300. After that the level of protein intake increases slowly, however, important shifts from vegetable to animal sources occur. The relationship between animal protein consumption and changes in per capita income is closer and more sustained throughout the entire income range than for total protein or any other nutritional indicator.[23]

The importance of the level of incomes and the changes in income over time have an important bearing on potential commercial markets for highly concentrated protein products. It also influences the role these products may play in future Food for Peace programs.

Implications of Economic Development

The diagrams (figures 2 through 6) raise many questions regarding the nature of world food needs and the manner in which they may be met.[24] Probably the most important is what are the opportunities and limitations for raising the nutritional level of food consumption?

In practically all countries this involves increased domestic production. In the developed and rapidly developing countries it also involves increased commercial trade. In the slowly developing countries domestic production is expected to increase but not nearly enough to meet the requirements of the expanding population. These countries are not in a position to increase greatly commercial food imports because of the shortage of foreign exchange. Consequently, the slow growth countries will continue to rely heavily upon food aid under Food for Peace type programs.

Economic development of the underdeveloped countries offers the greatest hope for reducing the world food deficit, which includes energy foods, fats, and proteins. As economic development proceeds, improvements in dietary levels are expected. The big question is whether the rate of development will be enough to meet both the increased needs for a rapidly expanding population as well as the increased demand for food that accompanies the increased personal incomes resulting from the development process itself. The charts (Figures 2, 3, and 4) illustrate the differences expected in the ability of individual countries to meet their increased food requirements as the development of their economies proceeds.

Inadequacy of World Food Distribution Systems

The marketing systems throughout most of the world suffering from hunger and malnutrition are generally inadequate to provide for the physical distribution of food from producers to those suffering from hunger. The degree of inadequacy increases as one moves from the rich or highly developed to the poor or slowly developing countries. In reality, the inadequacies of existing marketing organizations and structures in individual countries are to a large degree a reflection of the level or stage of economic development.

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[22] Ibid. (Fig. 16, p. 45).

[23] Ibid. (Fig. 18, p. 47).

[24] Figures 5 and 6 added a new component-the "economic" deficit due to rising income, which is of overwhelming importance as development proceeds.

Marketing systems in the more highly developed countries are dynamic and are capable of distributing large volumes of many different varieties of staple, fresh, and processed food products in an efficient manner to a wide variety of consumers. The pricing system in these countries reflect market conditions and provide the incentive for producers to shift as well as to expand production.

In contrast, the marketing systems found in the underdeveloped countries are not physically adequate for moving greatly increased quantities of food from producers to consumers or "would be" consumers. This is true whether the food is domestically produced or imported. In many cases an effective market for increased amounts of food does not exist because of the lack of consumer purchasing power. Without effective market outlets a highly developed and expanding marketing system is not economically feasible. Consequently, it is reasonable to conclude that the underdeveloped state of marketing and food distribution found in the food deficit countries is not incompatible with the existing level of economic development. This situation poses some important questions as to what the physical obstacles to distribution are as well as to how the problems of hunger and malnutrition may best be attacked.

Marketing systems vary from country to country, but on the whole their degree of physical inadequacy may be characterized as follows:

(1) Lack of storage facilities, including refrigeration,
(2) Lack of transportation facilities, both internal and external, including lack of port facilities,
(3) Lack of processing facilities,
(4) Lack of administrative and technically trained or experienced personnel, and
(5) Lack of initial as well as self-generating capital required for progressive market development.

The improvement and development of food distribution systems in the developing countries is dependent upon progress in world economic development. The extent to which the developing countries are successful in implementing a sound development strategy will depend to a large degree on the technical and financial assistance provided by the high income or developed countries of Europe, the United States, Canada, Australia, and Japan. This raises the question of how far the developed countries are willing to go in assisting the underdeveloped countries to move ahead in the development of their economies. The motivating factors for supporting world economic development embrace humanitarian, national security, and economic considerations. The Food for Peace program is based on both humanitarian and economic grounds, but the expansion of commercial market outlets for U. S. farm products above current levels is dependent upon the rate of economic development of the underdeveloped and populous areas of the world.

Considerations Relating to Efficiency of Nutrient Sources

Why is efficiency of protein sources important? What are the implications to research and to cottonseed protein concentrates?

The reason that least-cost sources of protein are so important is due to the world shortage of food protein. Further, in the years ahead the world protein shortage is likely to increase. Therefore, it is essential that resources be used as efficiently as possible in endeavoring to meet human protein needs.[25]

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[25] "Efficiency" is also used to describe the biological value of proteins.

There is no surplus or burdensome carryover of cottonseed and soybean meal today. There are no Government subsidies required to sell these commodities at current prices. If there were a demand for high protein concentrates for food purposes, either in the United States or in the export market, the food market would have to outbid the feed market for the available supplies. The countries where protein shortages exist, and are likely to continue in the future, represent low-income regions. In those countries, people do not have adequate purchasing power and their needs are not reflected in normal commercial markets. From an economic point of view these countries do not represent dollar markets.

Before arriving at implications to research and to oilseed protein concentrates as a source of supply, it should be helpful to review some of the problems and implications involved in measuring efficiencies of alternative protein sources.

There are several ways of measuring efficiency. Each method may be valid, depending upon the purpose for which it is used. For example, one method could be a physical measurement by which efficiency is expressed in terms of output per unit of input. We can measure protein yield per acre for the major crops produced in the United States. This relationship is shown in Table III.

This approach assumes that the cheapest source of plant protein comes from crops relatively high in protein having the highest yields per acre. Note that soybeans have the largest yield of protein per acre of the crops. They yield 60 percent more than the next highest yielding crop--dry peas--and 70 percent more protein per acre than potatoes However, physical yield alone may not provide an accurate measurement for determining the most efficient sources of protein because of differences in production costs between commodities.

A protein-yield-per-acre measurement also implies that a unit of protein is equal for each of the various sources. It is well known that not all sources supply protein equally balanced in amino acids. Also, it is recognized that a chemical measurement of protein may not accurately reflect a biological measurement. There may be some question as to whether digestibility tests based upon experiments using rats can be applied to humans with respect to amino acid requirements. We know that processing affects protein value. For instance, protein inhibitors in soybeans can be destroyed or inactivated by heat. So, processing can be used to add to or detract from protein value. An informative presentation of the relative merits of protein concentrates was given by Dr. Aaron Altschul at the Conference on Protein-rich Foods for Developing Areas, Rome, Italy, October 21-25, 1963. His report covers technical aspects of protein sources which must be considered.

When economic, social, and cultural considerations are brought into the picture, the measurement of efficiency becomes even more complicated. To mention a few--the customs, habits, and traditions of the various people affect the acceptability of protein supplied from different sources and the form in which it is acceptable.

Education on nutritional requirements, or lack of such education, affects the demand for protein from different sources. The per capita income level affects the price offered or demand for protein supplied from different sources as well as the form in which it is supplied. The cost of supplying protein also varies between sources and the form in which it is supplied. What does all this imply?

It means that in the market place, resources will tend to be allocated to maximize profits. Since many people have needs, but lack purchasing power, a cost-price comparison will not measure efficiency of protein sources in terms of need. Despite the

Table III. -- Per acre protein yields of the edible part of foods for specified crops

Commodity	Unit	Yield per acre average 1957-59	Protein yield		Rank
			Per unit[1]	Per acre	
			Grams	1,000 grams	
Food grains:					
Wheat, all	Bu.	23.5	3,292	77	8
Rice	Cwt.	32.3	2,686	87	7
Rye	Bu.	16.5	3,074	51	12
Feed grains:					
Corn	Bu.	50.1	2,090	105	5
Oats	Bu.	39.9	851	34	16
Barley	Bu.	29.6	982	29	17
Grain sorghum[2]	Bu.	34.2	2,090	71	9
Other food crops:					
Potatoes	Bu.	293.8	456	134	3
Beans, dry	Lb.	1,185.0	104	124	4
Sweetpotatoes	Bu.	118.5	385	46	14
Peas	Lb.	1,301.0	108	141	2
Oil crops:					
Soybeans for beans	Bu.	23.8	9,504	226	1
Peanuts, picked and threshed	Lb.	1,090.0	88	96	6
Fruit:					
Apples	Bu. [3]	130.0	58	7	19
Oranges	Cwt. [4]	181.2	290	53	11
Peaches	Cwt. [5]	123.3	200	25	18
Pears	Cwt. [5]	162.2	260	42	15
Grapes, American	Cwt. [5]	125.2	500	63	10
Grapes, European	Cwt. [5]	145.4	350	51	13

[1] Unpublished report by Robert O. Rogers, Assistant Director, Product and Process Evaluation Staff, Office of Administrator, Agricultural Research Service, U.S. Department of Agriculture, Washington, D. C. Protein yield calculated from Composition of Foods--Raw, Processed, Prepared, Agricultural Handbook No. 8, Table 2. Yield per acre, except as otherwise noted, from Crop Production, Annual Summaries, 1958, 1959, and 1960, Crop Reporting Board, Agricultural Marketing Service, U.S. Department of Agriculture, Washington, D. C.

[2] Used same protein per pound as for corn.

[3] Based on the 1944-46 average of 116 bushels and the 1975 projected economic attainable of 140 bushels.

[4] Two-year average; from Florida Citrus Fruit, Annual Summary, 1959.

[5] Average for 1956-58; from Crop Report and Related Data, Federal Reclamation Projects, 1956, 1957, and 1958, Table 6, All Projects, All Regions, All Divisions, U.S. Department of Interior, Washington, D. C.

worldwide shortage of food protein, cottonseed and soybean protein concentrates are exported as high-protein feed to Western Europe and Japan for livestock. These concentrates are not used in volume for human consumption. Since cottonseed and soybean food proteins require additional processing, it would increase the cost as a food over feed protein. Unless effective demands for oilseed protein concentrates can be developed, exports of cottonseed protein concentrates to low-income countries will be limited to shipments under food aid programs and should be justified on a political, military, or humanitarian basis.

For the slowly developing countries as represented in Figure 4, there is little evidence that commercial sales of high-protein foods can be developed before the requirements have been met for high-energy foods. Even in the United States some of our low-income families receive a poorly balanced diet. This indicates that economic allocations based on purchasing power and the profit motive will not allocate resources on the basis of human needs. However, before forming value judgements or suggesting implications, it would be well to attempt to proceed from facts as nearly as we can obtain them.

What are the cheapest sources of protein? A rough guide to the relative efficiency of protein sources can be obtained from the data presented in Table IV. The value of nonprotein ingredients in the sources has not been computed; however, the relative costs are indicative of the general relationship among cereal, dairy, poultry, meat, and fish sources of protein.

Note that the high-protein concentrates (soybean flour or grits and cottonseed flour) and fish appear to be the cheapest sources of protein. Meat and poultry are the most expensive. For convenience in comparing costs, it is possible to group the protein sources into 3 cost categories.

I. Less than 20¢ per pound of protein

Soybean flour or grits
Fish meal
Cottonseed Flour (Proflo)

II. From 20¢ to 60¢ per pound of protein

Whole wheat
Dry skim milk
Peanut meal
Bulgur
Wheat flour

III. Over $1.50 per pound of protein

Poultry
Meat

Appraisal of Potentials

The entire world population represents a potential market for new and improved protein products.

The type of product that best meets the desires of one group may be totally unacceptable to another. The type or kind of product that best complements the available food supply of one country may not be suitable at all to another.

Therefore, no new technology or technological approach for the development of new and/or improved protein concentrates should be ruled out at this time as being inferior to another. It would be a gross mistake if product cost or the relative efficiency of protein source materials should be singled out as the sole or even major criteria for judging the relative priorities for research and development of protein concentrates.

It is possible that the commercial market potentials for a high-cost specialty protein product may be greater in some countries than that of a low-cost protein concentrate Conversely, a low-cost protein concentrate for dietary supplementation may be in demand (commercially or for inclusion in food aid programs) in some countries while a higher cost product would be out of the question. Another important variable affecting the relative importance of costs as well as the source of proteins is the human factor, where the matter of preference and acceptability becomes highly important.

Table IV. -- Relative cost of protein from selected raw material sources

Protein Sources	Unit	Cost per unit	Protein	
			Content	Cost per pound
		Dollars	Percent	Cents
Cottonseed flour (com. product)	Lb.	[1] $0.097	57	0.17
Soy flour (food quality)	Lb.	[2] 0.054	52	0.11
Fish meal (feed grade)	Lb.	[3] 0.10-0.12	70-85	0.14
Wheat (Kansas City)	Bu.	[4] 2.20	12	0.30
Dry skim milk	Lb.	[5] 0.144	35.6	0.40
Peanut meal (defatted)	Lb.	[6] 0.25	55-60	0.42-.45
Bulgur	Lb.	[7] 0.0565	12	0.47
Wheat flour	Lb.	[8] 0.066	11	0.60
Chicken (dressed)	Lb.	0.30	20	1.50
Beef (retail)	Lb.	0.80	18	4.44

[1] Traders Protein Division of Traders Oil Mill Company, Fort Worth, Texas. Carlot f.o.b.

[2] Defatted soy flour, f.o.b. Decatur, Illinois, 50-thousand-pound-minimum carlots, $5.40-$5.50 per cwt.

[3] Price obtained from Charles Butler, Fish and Wildlife Service, United States Department of Interior, Washington, D. C.

[4] December 24, 1963 quotation in Wall Street Journal.

[5] Statistical Services Section, Farm Income Branch, Economic Research Service, United States Department of Agriculture, Washington, D. C.

[6] Southern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, New Orleans, Louisiana. Trip Report S-63-149, June 6, 1963.

[7] Gulf Port prices for Houston and Galveston, Texas, provided by Arnold Dahl, Agricultural Stabilization and Conservation Service, United States Department of Agriculture, Washington, D. C., December 26, 1963.

[8] December 24, 1963, quotation in Wall Street Journal, Flour, hard red winter, New York.

Variables such as these must be kept in mind in appraising the future markets for protein concentrates.

Developed or High Income Countries

The most important commercial markets for specialty or highly processed food products are found in the higher income groups in the higher income countries. The higher income groups are generally overfed in terms of carbohydrates, fats, and proteins. A favorable market for specially prepared protein concentrates exists in the developed countries if a product can be developed to meet consumer demands. Such a product is needed to meet the demands of the obese. The price of such a product would be secondary to its acceptability.

On the basis of average consumption levels or average availabilities the developed or higher income countries of Europe, the United States, Canada, and Australia do not indicate important deficiencies in protein. Do not let the averages mislead you. Even in the higher income countries there are low-income groups that are potential commercial purchasers of low-cost protein concentrates for use as dietary supplements.

For the market as a whole, however, the protein consumption in these countries is heavily weighted with the more expensive animal products. Consequently, costs or prices of specially prepared protein concentrates are not a major obstacle in the development of commercial markets. In fact, quality and promotion probably would be the overriding factors determining the commercial success of specially prepared protein products.

It should be observed that the situation in the developed countries is not incompatible with good sound economics. The markets in the advanced high-income countries justify the use of more expensive sources of protein in the human diet just as the resource capacity of these countries is sufficient to support a higher standard of living.

Moderately to Rapidly Developing Countries

The moderately to rapidly developing countries probably offer the most attractive commercial market potentials for high-protein concentrates. At the present time grains and pulses supply a large share of the protein consumed in these countries, with relatively small amounts being supplied from animal sources. Many of these countries do not have the agricultural resource capacity to rely very heavily upon proteins from animal sources in the future.

As the moderate to rapidly developing countries develop their economies and as foreign exchange earnings increase, their ability to import specialty food products to meet identified nutritional needs is expected to improve. The higher income groups in these countries will provide a reasonably favorable commercial market opportunity for both highly processed specialty protein products as well as moderately priced protein concentrates for use as a dietary supplement. The lower income groups of the population will still be limited in their ability to supply their protein needs adequately from the commercial domestic market and will continue to rely heavily on grains, root crops, and pulses. The extent to which these groups become important consumers of high-protein concentrates will depend on government assistance to eliminate identified dietary deficiencies or the food aid programs under P. L. 480 or the World Food Program.

These nations as a whole will be "cost conscious" in regard to food consumption and nutrient sources. Although the higher income groups will be less cost conscious than the lower, the relative costs of high-protein concentrates will have an important bearing on how extensively they will be used to reduce dietary deficiencies in the lower income groups within a country.

Poor or Slowly Developing Countries

The poor countries or the slowly developing nations are expected to continue to have the greatest difficulty in meeting their nutritional requirements, as illustrated in Figure 4. It does not appear that the nutritional deficit can be closed in the distant future by increased domestic production and commercial imports because of the fact that progress in economic development is expected to be slow and not enough to overtake the increased food needs of the expanding population. Inadequate earnings of foreign exchange coupled with the high priority of capital goods and other essential imports will continue to limit the ability of these countries to import sufficient food to meet the basic nutritional requirements. It is not likely that these countries will be able to import large quantities of even the low-cost high-protein concentrates. Even though large protein deficiencies exist, first priority for food imports will be given to the cheapest grains to meet the energy food requirements of the population.

A small commercial potential for high-protein concentrates may exist in these countries, but it will be limited primarily to government purchases for specifically identified purposes to meet specific nutritional goals.

On the other hand the need for low-cost high-protein concentrates for use in food aid programs will be great in these countries as more attention is placed upon closing the "nutrition gap". However, the emphasis on high-protein concentrates must be balanced off against the priority of meeting the need for basic energy foods. These needs in most situations likely will continue to be overriding under food-aid programs.

Summary

In summary, it is apparent that the greatest commercial market potentials for high-protein products likely will be found in the industrial or high-income countries and the rapidly developing countries where individual incomes are already high or are increasing. Limited commercial potentials may exist in the slowly developing countries.

The relative importance of protein costs increases as one moves from the developed or higher income countries to the low-income or slowly developing countries of Asia, Africa, or South America. In the high-income countries quality and promotion will probably be more important than price to a large segment of the population.

In regard to the inclusion of low-cost high-protein concentrates in food aid programs, it is apparent that they would be limited primarily for use in the slowly developing low-income countries and maybe to some extent in the moderately developing countries. The relative cost of these products per unit of usable protein would be highly important though not overriding. Extensive use of high-protein concentrates in food aid programs would depend largely upon organized efforts to attack specific nutritional problems in particular situations. Along with relative costs, the source of protein as well as its general acceptability by local people must be taken into account.

DISCUSSION

L. E. Allen: I would like to withhold any discussion or questions at this time, even though there are many controversial subjects that have been presented and which could be further discussed, and call on Mr. Louis Bean, a philosopher.

COMMENTS

by

Louis H. Bean
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Mr. Chairman and Friends: I don't know who gave the chairman the privilege of changing my profession. I have been called all kinds of things, a political forecaster or stock market forecaster, sometimes an agricultural economist, statistician and whatnot. And here I am supposed to perform as a philosopher. As your philosopher, of course, my field must be the whole range of human activity and all problems of the universe. And I am supposed to cover all that before you get hungry, which of course I will not attempt to do.

I shall limit myself to bringing out only a few points in the paper that you have just heard. It is an historic event in the field of agricultural economics to listen to this paper. I think it is the first time that the responsible economic researchers, researchers in the U. S. Department of Agriculture, have undertaken to bring themselves up-to-date on the developments and the potentialities in the field of oilseed foods. The paper, of course, is an excellent paper because it is authored by two experts of the Department of Agriculture, and so in one sense, there is little that I could add or disagree with, particularly disagree with. I would, however, like to comment on two or three points and then perhaps suggest some additions which I would like to see in a second paper to follow this one at the next appropriate occasion.

Take for example, the figures given you with regard to global aspects of the oilseed food market in terms of the world food deficits as estimated recently by the Department of Agriculture. They have been given you in terms of dollar figures represented by the three or four categories of deficits, commodity categories. When these estimates were presented you were told I would make some comments about them or add to them. Well, that wasn't mind reading on the part of the authors of the paper. It refers to a bit of work that I in fact asked them to perform for me. I wanted an expression of these world deficits not only in dollar terms but also in terms of the particular commodities and quantities that you are interested in, namely oilseeds.

They therefore, supplied me with some figures which represent the protein value in the deficits if they were all to be met by soy flour. I believe the figures run to well over a billion dollars if the animal protein deficits and the other deficits indicated in the paper are met by soy flour alone. So I hope that possibly as a footnote to this paper when it is finally submitted for printing, or on another occasion, these deficit figures will be spelled out in terms of the various oilseed products in the world deficit. That would give us a more tangible global expression of what the market for oilseed products might be--not that there is any hope that oilseed products will soon fill these gaps.

I am tempted at this stage to comment on a remark that was made by Dr. Milner in response to a question I asked. I had a purpose in asking it and it bears on one of the points in the paper just presented, the profitability problem in listing the potential markets for oilseed foods. One gets the impression that progress is likely to be slow because private enterprise doesn't see an early return for investment. You may recall that Dr. Milner said that one of the things proved by the Rome Conference last October, was that there are corporations who are willing to postpone profits on their investment, thus probably expressing their humanitarian desire to further the expansion of this field. This is very encouraging because the comments I have picked up in asking people what the prospects were have always been sort of negative, that private interests are not going to move into this field rapidly because they do not see profits coming soon enough.

Much was said in the paper with regard to the necessity of economic development in the undeveloped countries where the bulk of the world's malnutrition problem exist. Again, you get the impression that progress is going to be slow because unless you have increased per capita incomes, the market for these products will not develop. I am inclined to think this is too pessimistic a view. In the first place, in the expansion in world demand for food there are two elements, the effect of the rise in population and the possible effect of increasing per capita income. Well, I suggest that we need not wait for the increase in per capita income. The increase in population alone opens up additional markets for oilseed foods.

With regard to the problem of economic development as a basis for further extension of investments in this field, I would like to point to some basic economic development arithmetic. Say we are looking forward to the time when the per capita income of India, and other undeveloped countries, low income countries, will be doubled. Now doubling of per capita income would mean a great deal to those interested in markets. But are we likely to soon see a doubling of per capita income in a low income country? Turn back 70, 80, 90 years in the United States and you will find that the United States then was at the industrial stage that the undeveloped countries are in today.

How long did it take us when we were 70 percent agricultural to double our per capita income? The United States, with all its resources, with the influx of population, with technology, with foreign capital coming in here, how long did it take us to double our per capita income in those days? It took us approximately 20 years, and we accomplished that expansion in economic growth as a result of shifting the proportion in agriculture from say 70 percent down to 50 percent. We again doubled our per capita income when we later shifted from 50 to 30 percent agriculture. And if you take this simple measure of a stage of industrialization in relation to the per capita income for different countries, you will find it a fairly good rule to go by in shaping expectations. Recently I had this verified when I studied the progress made in Mexico. Mexico went through a revolution before World War I as many of you know. Their economy has expanded; their per capita income has increased; and their proportion in agriculture, their stage in industrialization, is reflected in the fact that they have dropped from a 70 percent agricultural country to approximately 50 percent. Their per capita income during this span has doubled, and it didn't come overnight. It came over a span of something like 20 years.

So I would suggest that in further papers in this field we do not dwell solely on the slow progress of economic development, but take a deeper look at the potentialities of marketing products today. There are dedicated food specialists in the world, there are corporations that are smart enough, I think to devise the processing methods necessary to reduce costs so that low income people can buy.

The second author of the paper, Mr. Bitting, talked to you about the relative efficiencies in the resources for the production of protein. I hope that in his second paper covering his work he will develop another aspect of the supply of proteins. I think that the shortage of proteins today is not quite as much as indicated. Why do I say that? If you take a look not only at the sources from which our proteins come, cottonseeds, soybeans, etc. but also at their uses, you see an amazing situation. In India, it has been pointed out, much of the cottonseed is used for fertilizer, for feed. Their local supply of oilseed products which runs close to two million tons is used mostly for feed and for fertilizer. Very little goes into food uses. But there you have a source of protein which could be made available if not today, tomorrow, for human needs. It is now, I think, being inefficiently used, wastefully used in view of the inadequate food protein supply. I am not an agriculturalist, my farming always has been done on top of a desk. So what I am about to say may not make sense, but I have picked up these notions from people who know. It seems to me that it doesn't make a great deal of sense for us to encourage India to use its meal for livestock. I understand that meal can be used to feed dairy cows, buffalos. Well, I understand from some of you that it is possible to make milk out of soybean in which case you get the full value of the proteins in your meals, whereas if you process this through the cow, you get a fifth or a fourth--I don't know what the figure is, but it is a small proportion of the total food value that goes into the animal. India, with 450 million people, and 200-300 million animals has to supply for these 700 million living creatures, man and animal, out of a common resource--the land.

I suggest that if the feeding of animals wastes 75% of potential food proteins and if technologists can show India how to convert its meal directly into food and milk, then I think we will have licked what to me seems to be a major inefficient operation. I think the same situation exists in Brazil where a third or two-thirds of its use of cottonseed goes into fertilizer and feed. And so, the deficiencies in this field are not merely in resources or production, but in the way the resources or the production are actually put to use.

Now there is another aspect to the inefficient use of the oilseeds in a country like India, the need for processing machinery. To buy machinery one must have dollars. Well, I suggest that if India could convert the oilseeds into meal for the foreign markets, that is instead of using it as fertilizer, then they would have additional foreign exchange which they could divert to purchase food processing machinery.

On the use of cottonseed for feed, I am advised that it is possible to substitute a chemical source of nitrogen, say urea for cottonseed. I understand that experiments conducted at Beltsville, Maryland, indicate that you can get good results by substituting urea for cottonseed. If India could substitute a cheaper product like urea in its feed instead of cottonseed, and use the cottonseed in its various byproducts in the export market, it would have additional foreign exchange. And the figure for India is quite substantial; it runs to 150 million dollars of products that are now being used uneconomically and inefficiently. If only a portion of that 150 million dollars worth of products could be diverted in this way and converted into foreign exchanges I think India's progress in closing its nutritional gap would be much faster.

Now, I would like to comment on the fact that progress in this field is being held back by a number of roadblocks, one of the major ones, I think, being a lack of public knowledge, even at the highest levels of administrative responsibility, as to what is going on in this field. May I give you two examples at the risk of holding you longer than I should.

I recently had occasion to prepare a report on the possibilities of using soybeans for soy milk for the office of the Secretary of Agriculture. The Secretary's Office involved in the Food For Peace Program and in foreign economic development, wanted to be updated on the use of soybean milk that it had recently heard about.

I discovered much literature on the subject stretching all the way back to 1900. There were many, many articles from the USDA laboratories full of information on developments in soybean use. And yet responsible people at the top of the government in the Food For Peace Program in the Department of Agriculture, and the foreign aid program, were not aware that it has been possible for some years to go to the drugstore and buy soy milk. True, you pay a fancy price, but it is there.

That is observation number one. The other has to do with a former Secretary of Agriculture whom I think many of you know, Henry Wallace. Back in the 1930's, he was interested in soybeans, and a thousand and one other things, in fact he had the Department people living on soybean products for a week. In the Department's lunchroom we had toasted soybeans, and soy butter and soy milk. So I felt that certainly he would be sympathetic and up-to-date on the potentialities of soybeans as a source for milk. When he found me working on this report he said, in effect, "Well, I don't think you will find much. As I remember, I tried to find out something about it, tried to get our Home Economics people to develop a palatable milk, and they didn't get anywhere".

I said, "Well, in that case, come up to my room".

It so happened that I had in my desk a can of Soylac; the first, I believe, of the soy milks made out of soybeans in the United States. I stirred some of the powder into a glass of water and handed it to him. He tasted it and said, "My goodness! They have succeeded in removing the bean flavor, haven't they?"

That afternoon when he gave one of the Centennial talks at the Department he announced that he had been brought up-to-date on soy milk developments. Well, if intelligent men like Henry Wallace, if top officials in the government are not aware of current exciting developments in oilseed foods, then I think that the potential export market, the potential uses in the United States are being held back by that lack of official knowledge and public understanding. I would recommend that something be done about it. I am really serious about this because I have observed the retarding effect.

I am often shocked at running into prejudices, fears, and presumed facts which are not so. Take the simple controversy of the preference for animal protein over vegetable protein. Now, I don't know where this Conference stands on that great issue. As for me, the issue was settled in two ways: at the 1961 Peoria Conference on soybeans when Dr. Sebrell announced that the latest scientific discoveries indicate babies can be fed on vegetable food entirely, without animal protein at all; and by looking through the first page or two of Genesis.

Now, I realize an economist doesn't often refer to the Bible but those of you who are students of the Bible know that when God created the universe he created grasses and animals. And he said to the animals, "The grasses shall be your meat." This was instruction to the animals. He created man and seeds and fruits, and to man he said, "These shall be your meat." That is why I am interested in having soybean and cottonseed and other things processed into flour, milk, and meat substitutes. The Bible wants you to do that, and Dr. Sebrell and other top nutritionists say it is all right!

There are a number of public relations problems that cottonseed and soybean people will have to overcome. You know, there are people in the United States who don't dare to use the word soy milk. They prefer to say soy beverage. Why? Because they see behind them the influential dairy lobby. If they say soy milk, they know that they are confronted with another battle like that involving margarine, and they don't want to get involved now in a milk battle. This is realistic. If you want to learn of the problem Dr. Harry Miller had in creating the first soy milk called Soylac, read it in the book about him entitled "China Doctor." Back in the 1930's he went to the Medical Association for approval of his formula. They approved it, but told him not to call it milk, to say that it was a substitute for milk; so knowing that the Romans called milk "lac" he called his product Soylac. This is an indication of one of the problems, and there are many such that retard the prospect for domestic and foreign use of oilseed food products.

Let me hurry to a final point and that is that when these authors come to prepare their second paper, and I will give them presently the occasion when they should do this, they should go beyond their essentially theoretical description of this field into an examination of the tangible things that I can see going on. Here are some of the things that I know are available for examination. I don't say that I approve of all these things because I don't know enough about them, but they seem to me quite exciting and economists ought to take the next step and thoroughly familiarize themselves with each of the items I am about to list.

You will presently hear a great deal about Incaparina, so I am not going to touch on that. But there is an opportunity for combining cottonseed products with soy products into formulations which can be made available, I take it, not only in Latin America, but in the United States and elsewhere. I hope this subject will be opened up to us in great detail.

I am told there are dairy interests in Brazil, who would like to have a continuous supply of milk to cover the period of short production. Their problem is not having enough in the low season. Our problem is having too much in the high production season. Perhaps the deficit can be met by combining soy milk with regular milk.

I can visualize interesting markets for American soy flour. I don't know whether cottonseed flour fits into this or not. I understand that in India where they know how to make milk out of peanut flour, there has been a great deal said, much perhaps done experimentally in combining wheat flour and soy flour. The potentialities here strike me as rather interesting and probably quite large, and call for the economists' attention.

With regard to soy milk, I happen to have received just before coming here a letter from Dr. Harry Miller, whom I referred to before as the originator of Soylac in the United States about 30 years ago. I suppose you all know Dr. Miller and his work. He has established some small soy milk plants in several countries of the Far East. He wrote me about the commercial operation that is going on in Hong Kong. A Mr. Lo to whom he turned over his formula for soy milk about 15 years ago, is today running a thriving business. A year or so ago I read that he undertook to double his operation, which means that it is a profitable business. Dr. Miller says that soy milk today is being sold there in larger quantities than all other beverages combined. Well, my suggestion to the USDA economists is that they investigate this field and make it known to the public because ultimately it is bound to benefit agriculture, the oilseed industry, and the public. Now these exciting, emerging things remind me of a line in poetry, "My heart leaps up when I behold a rainbow in the sky." Well, these exciting prospects are rainbows as far as I am concerned; I would like to see someone catch hold of them. I think the simplest way to catch hold of them would be for the economists to do what you technologists have been doing now for several years. You meet annually. You talk about technological problems. I think that the economists should start a series of annual conferences dealing with the economic aspects of oilseed foods and the potentialities that I have hinted at. In that way, I think our hopes for closing the world's nutritional gap would come to realization much sooner than they would otherwise.

ANATOMY OF COTTONSEED : CRYOSTAT FROZEN SECTIONS IN SEED ANATOMICAL STUDIES

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Introduction

In seeds which produce large quantities of oil, protein content is relatively high, and starch content low; cottonseed is almost totally devoid of starch, its energy reserves being confined to oil and protein storage products. New attempts are being made to clarify the anatomy of oil seeds as a prerequisite to investigating the metabolic systems which control the production of protein, and to elucidating the role of such cell inclusions as protein bodies, phytin globules, and lipid droplets, in metabolic processes. A central problem in cottonseed investigations is the site of lipid deposition within the mature seed. It is not known whether the oil occurs in specific subcellular organelles, or whether it is so closely associated with the protein as to be unidentifiable as free fat. Conventional methods of botanical histology have never been satisfactory for locating the sites of residual lipids because of the difficulty of sectioning oil seeds without mechanical displacement of the oil which is liquid at room temperatures. For this reason freezing methods have been advocated. The purpose of our effort was to explore the use of freezing techniques for investigation of oilseed anatomy.

The freezing microtome, using CO_2 as a refrigerant, has been used for many years in botanical histology. However, section quality is not adequate for most critical studies because of inconsistent section thickness, freezing artifacts and the loss and movement of cell constituents. The introduction and perfection within the past few years of the cryostat (cold microtome) has now made it possible to obtain superior frozen tissue preparations which often excel preparations made by other methods. Cryostat frozen sections are widely used in animal histological studies, the techniques having been largely perfected in medical research laboratories (Russell et al., 1961).

In spite of its great promise, cryostat fresh frozen plant material has been used by only a few workers, e.g., Chayen et al. (1960) and Fried and Franklin (1961). In fact, Jensen (1962) has stated in a recent text on botanical histochemistry that, "... so little work has been done with fresh frozen plant material sectioned in this way that it is not possible to recommend a best procedure for handling the sectioned tissue."

In spite of the paucity of perfected research methods using frozen plant materials, this approach appeared to hold the greatest promise for histological investigation of sites of lipid disposition, within the mature cottonseed.

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[1] The research was conducted while the author was holding a summer (1963) appointment as Botanist in the Microscopy Investigations Section, Cotton Physical Properties Laboratory, Southern Utilization Research and Development Division, U.S. Department of Agriculture, New Orleans 19, La.

Materials and Methods

Cottonseed of the Acala 193 glandless strain were used in this study. Before the seeds were fixed and sectioned, the hulls were removed with a razor blade. This investigation was thus restricted to the embryo of the mature cottonseed.

Fixation. The embryos were fixed in neutral formal-calcium (Baker, 1946) for 6-24 hours. Both whole and small pieces (about 3x3mm) of embryos were fixed, depending upon subsequent use of the material. This fixation gives good stabilization of lipids if the tissues are not later subjected to harsh organic solvents. Lipids are stabilized enough so they do not move in aqueous solutions or in mild (50%) alcoholic stains (Jensen, 1962).

Infiltration and Embedding. Embedding is seldom resorted to in cutting frozen tissue though infiltration is not uncommon; polyvinyl alcohol, for example, is sometimes used (Chayen et al., 1960).

One aspect of this investigation required that entire embryos be sectioned in median longitudinal plane. It proved impossible to obtain coherent sections with the cold microtome using water or polyvinyl alcohol mounted embryos. The reason for this is that the cotton embryo is a friable structure, being largely composed of two much pleated and folded cotyledons. When sections are cut, the many folds of the cotyledons fall apart upon thawing on the slide.

Coherent sections were eventually obtained using a method devised by Lillie (1954) involving gelatin infiltration (15%) into previously fixed embryos under 20 inches vacuum for 16 hours at 38-40°C. After infiltration, the embryos were cast into gelatin molds, blocked, trimmed and quick frozen.

Quick Freezing. Three methods of freezing were used in this study. The first was used for gelatin blocks and the second and third for small pieces of fixed tissue.

1. Gelatin blocks were attached to cutting blocks as the latter sat on the mechanical quick freezing platform of the cryostat. Freezing time for the blocks was 3-5 minutes, a time far too long for good preservation of intracellular details. However, this method of freezing was satisfactory for gross embryo study.

2. Intermediate freezing temperatures were obtained by using dry ice and acetone as a coolant for a freezing bath of isopentane. The isopentane was placed in a small beaker and this lowered into the dry ice-acetone mixture. Small bits of tissue were thrown directly into the cold isopentane where they froze within a few seconds. The temperature of the freezing bath was about -65°C.

3. Extremely low temperatures were obtained by using liquid nitrogen as the coolant in conjunction with an isopentane freezing bath. The isopentane bath was cooled to slightly above its freezing point of -165°C. and bits of tissue were thrown directly into it. There was no apparent superiority of method 3 over method 2 in this material. The relatively low moisture content of the tissue could account for the lack of clear superiority of extremely low temperatures in quick frozen cotton embryos.

Sectioning. All sections were cut on a Minot rotary microtome housed in an International cryostat maintained at -20°C. Gelatin blocks were cut at 14μ and uninfiltrated pieces cut at 10μ thickness.

Histochemical Methods

1. General Histological Staining.
   a) Longitudinal sections of entire embryos were stained in Mayer's haematoxylin for 20 minutes. It was not possible to obtain good differentiation of nuclei with this stain. In this study the best that can be said of this stain is that it is a moderately satisfactory histological stain.

b) Chlorozol Black E (1%) in 50% ethyl alcohol was used as a histological stain for sections of small pieces of embryo tissue. Staining time was about five minutes with differentiation in 30% ethyl alcohol.

2. Lipid Localization. The methods which might be employed in lipid localization are conveniently grouped into so-called physical ones, which depend upon the selective solubility of colorants in the lipids and chemical methods based on chemical reactions which produce colored reaction products with lipids (Cain, 1950). Only the physical methods were employed in this investigation and the different procedures were as follows:

a) Saturated solution of Sudan IV in 60% triethylene phosphate (Gomori, 1952)

b) Saturated solution of Sudan Black in 60% triethylene phosphate (Gomori, 1952).

c) Saturated solution of Sudan IV in pure propylene glycol (Chiffelle and Putt, 1951).

d) Saturated solution of Sudan Black B in pure propylene glycol (Chiffelle and Putt, 1951).

e) Saturated solution of Sudan IV in 50% ethyl alcohol (Baker, 1947).

Mounting. All sections were mounted in glycerine-jelly.

Results and Discussion

1. General Anatomy. The cottonseed is nearly all cotyledon. A 24-hour-old embryo from a germinating seed (Figure 1) shows the typical dicotyledon morphology of an elongating axis with 2 attached cotyledons. That part of the axis above the attachment of the cotyledons is the epicotyl from which the seedling stem will develop; that below, the hypocotyl, the lower end of which bears a more or less developed primordium of the root. Within the ungerminated seed (Figure 2), the cotyledons are very much folded and convoluted, and occupy almost the entire seed cavity. There is no detectable endosperm layer except for a trace at the periphery of the seed.

2. General Histology. Frozen sections revealed many spherical bodies tightly packed within the spongy, palisade and epidermal cells of the cotyledons and within the parenchymatous cells of the hypocotyl (Figs. 3-6). These bodies are about 3-5μ in diameter. Such bodies have been described by Yatsu and Altschul (1963) to contain 28% lipids and to be high in protein. The spherical bodies were found tightly packed in all cotyledonary cells except those of the procambium. These bodies were also found in great abundance in the upper part of hypocotyl; diminishing in frequency in the more distal region of the hypocotyl.

The intercellular spaces between parenchyma cells were not very large in these preparations and this finding does not agree with that of Leahy (1948) who found large intercellular spaces. It is suggested that this discrepancy is caused by differences in the methods of tissue preparation; frozen sections are known to produce preparations with less shrinkage and such sections have greater density than do paraffin infiltrated preparations.

3. Lipid Localization. None of the Sudan colorant procedures produced definite coloration of lipid sites within any cells. In cases where tissue damage resulted in crushed cells, free lipids were liberated from the cells and gave a positive coloration with the colorants. The Sudan colorants did produce a faint diffuse coloration of undamaged tissues but this could not be interpreted as lipid site coloration.

The failure to obtain lipid localization with the Sudan methods would seem to mean that free lipids do not occur in the cells in a non-solid state, but are associated with other cell constituents in a bound state, in particular in membrane systems. It must be borne in mind that the Sudans produce colored lipid products by diffusing from a carrier (ethyl alcohol, propylene glycol or triethylene phosphate) into non-solid lipids (Cain, 1950). They, therefore, cannot produce coloration if lipids are not present in a free non-solid state. These findings are in agreement with Leahy's (1948) observation that free oil is not present within cottonseed cells and "... appears to occur within the cell as cytoplasmic emulsion".

Conclusions

The cryostat microtome permits slicing of oil-containing seed to give sections approximately 10 microns in thickness, which are adequate for study of gross anatomical features at the magnifications of light microscopy. However, more rapid freezing of the tissues at lower freezing temperatures is required for better preservation of cells. This appeared to be as well achieved at -65° with dry ice and acetone as at -165° with liquid nitrogen. Conventional staining methods did not reveal any accumulation of oil within the cell, except in specimens which had been mechanically damaged in preparation. The general conclusion from these observations is that the oil in cottonseed does not occur in the free form, but exists within protective membranes, or is bound to other cell organelles in such a way as to be unable to respond to coloration by oil soluble dyes.

Acknowledgement

The author is indebted to Miss Ines De Gruy for several of the photomicrographs and to Miss Mary L. Rollins for advice given during the course of this work.

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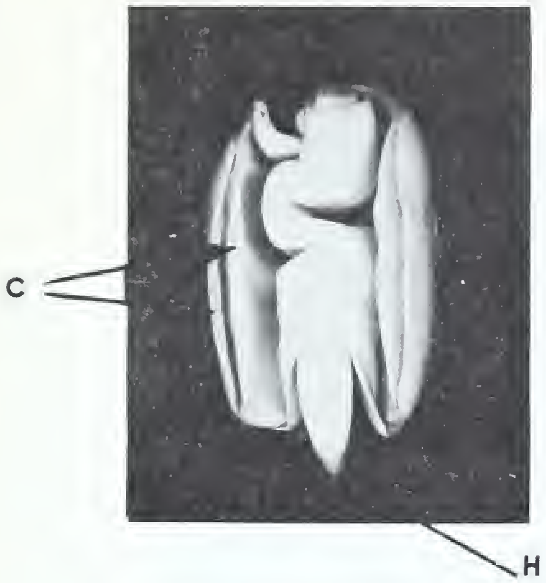

Figure 1
Photomacrograph of a 24-hour germinating cotton embryo. The two appressed and folded cotyledons are marked (C), the elongating hypocotyl (H). Approx. 6x.

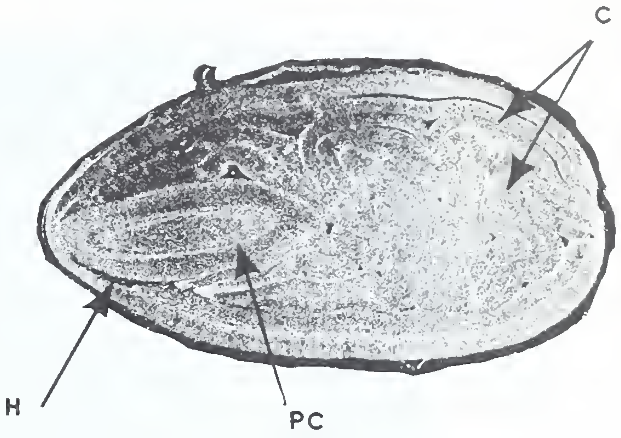

Figure 2
A near median longitudinal section of a glandless cotton embryo. All tissue shown is either cotyledonary (C) or hypocotyl (H), except for a peripheral trace of endosperm. The procambium is marked (PC). 10x.

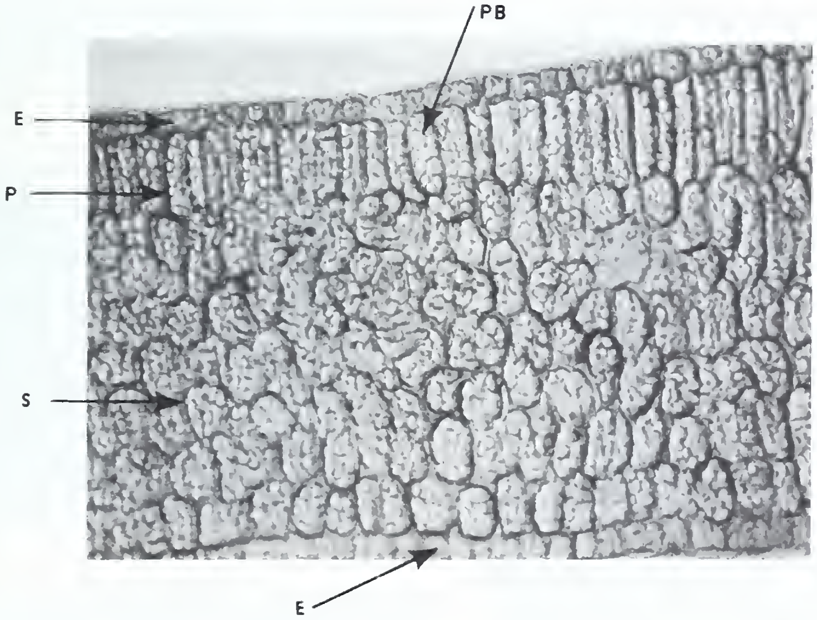

Figure 3
Section of cotyledon of cotton embryo showing epidermis (E), palisade layer (P), spongy mesophyll (S). Cells are filled with proteinbodies (PB). Fixed in formal-calcium, sectioned at 10u on a cold microtome, stained with Chlorozol Black E.

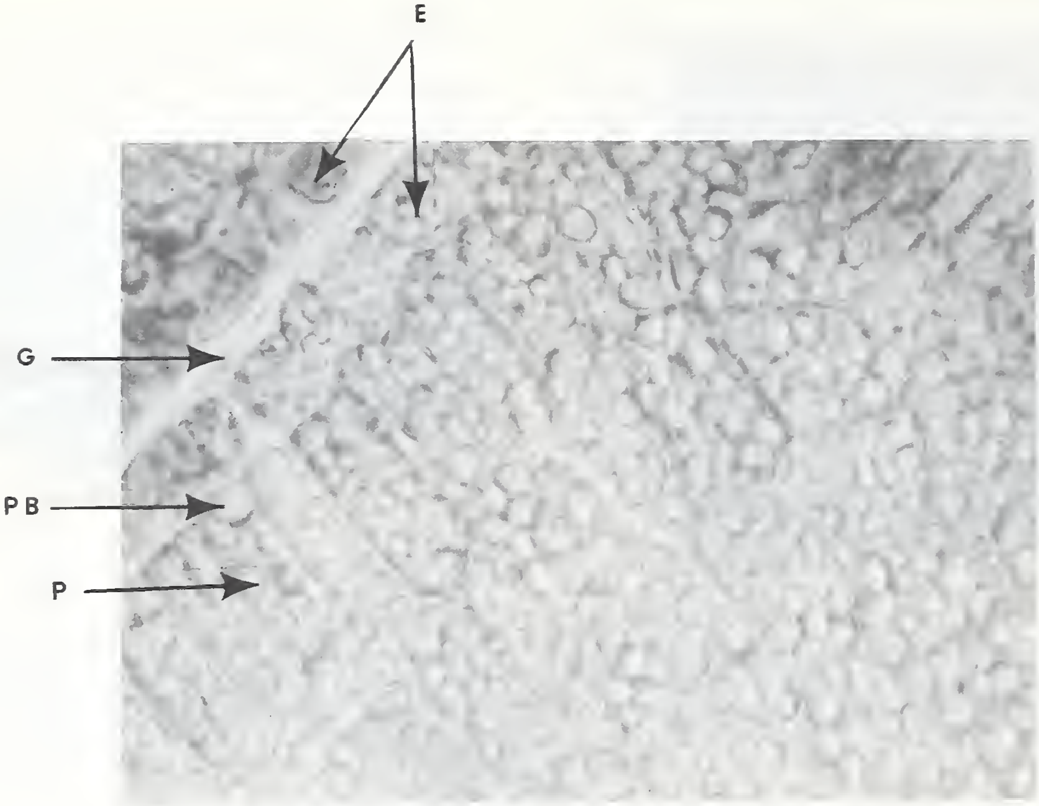

Figure 4
Section of cotyledonary tissue of cotton embryo showing portions of two appressed cotyledons with a slight gap (G) between the epidermis (E) of each. The epidermal (E) and prominent palisade cells (P) are filled with protein bodies (PB). All microtechnique data are the same as for Fig. 3. 1100x.

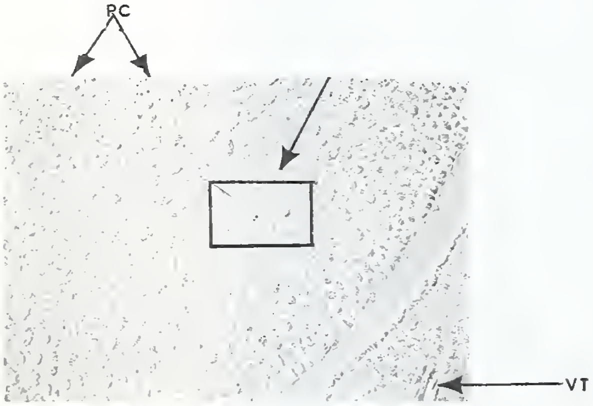

Figure 5
Cross-section of hypocotyl region of cotton embryo and a small portion of first fold of cotyledon in the lower right of figure. Hypocotyl procambium is marked (PC), cotyledonary vascular tissue is (VT). Fixed in formal-calcium, sectioned at 10μ on a cold microtime, phase contrast, no stain. 160x.

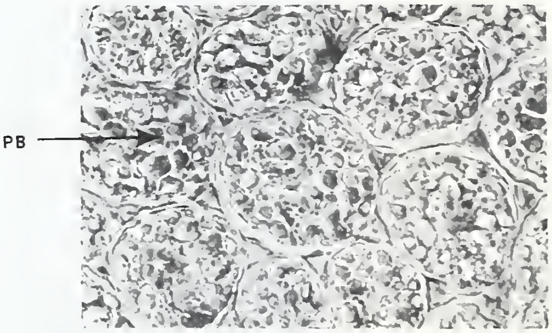

Figure 6
Cross-section through cotton embryo hypocotyl showing cells tightly packed with protein bodies (PB) of variable size. All microtechnique data are the same as for Figure 5. 1040x.

ULTRASTRUCTURE OF THE COTTONSEED

by
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It is becoming increasingly clear that the major portion of seed proteins, "storage proteins", if you will, are found in packets known as protein bodies or aleurone grains. Recently we have been able to isolate from the peanut seed protein bodies which contained almost pure seed proteins, divorced from the machinery protein of the cytoplasmic material. As part of this study, histological and cytological studies have been carried out on the electron microscope level. This talk deals with the ultrastructure of protein bodies and their relationship to other cytological entities. Since the cottonseed is an oilseed, the ideas evolved during this study concerning the site of oil storage will also be discussed.

At the light microscope level the bulk of the cottonseed tissue cells can be seen to be filled with myriads of spherical bodies. The bodies range in size from 1 to 5 microns in diameter. Electron microscopic studies using both osmium and permanganate fixatives coorborate this observation. The spherioidal bodies are interspersed within a three-dimensional network which fills the dry cottonseed cell. The bodies appear homogeneous, with no membranous material within them. They are surrounded by a single membrane and are filled with the "storage protein" of the seed. Usually they contain one to several holes, known as globoids. The globoids contain a substance which is reticular in nature. Various membranous structures and organelles have been observed within the intracellular network material. The network material is interpreted as dehydrated cytoplasmic material.

The space surrounded by the interbody network, has been named "spherosome" in the literature. It is believed that the spherosome is the site of oil storage in oilseeds. Under osmium fixation the cottonseed spherosomes appear as electron opaque areas closely resembling areas denoted as "lipid bodies" in electron microscopic literature.

In a previous communication from this laboratory, it was stated that subcellular particles containing 26% and 44% lipids were isolated from the cottonseed. Electron microscopy reveals that the 26% fraction corresponds to the aleurone grains and the 44% fraction corresponds to the fragments of the intercellular network material. It would seem that although phospholipids and certain other lipids are associated with the protein bodies, the major site of oil storage in cottonseeds is in the spherosomes.

DISCUSSION

M. L. Anson: Is there any comment or question in connection with the last paper, please?

A. M. Altschul: I would just like to underline the work of the two papers presented by Doctors Wall and Yatsu. I think that we can now say without qualification that the uniqueness of seed proteins lies in the fact that they occur for the most part in subcellular packages. The seed is a unique tissue in that it synthesizes large quantities of protein and keeps them in packages. These are the unique proteins that Yatsu called storage proteins. It is equally likely that the lipid occurs in the packages. Some of them may be in the spherosomes that Yatsu mentioned and some of them may be in the protein particles. You have a tissue which has very neatly packaged a lot of things. It has packaged lipids and it has packaged proteins. It has packaged gossypol; it might package other minor constituents. Commercial practice involves grinding up the seed, smashing it to bits, cooking, extracting, and pressing it, and then trying to separate the various constituents--minor and major. I think this is the easiest thing to do, but in the light of what these studies are showing, one might well wonder whether in the future this will be the best thing to do.

C. F. Lewis: I just wonder if you had a slide where you cut across what we are calling gossypol glands, to have a detail of its anatomy as you had other cells.

L. Y. Yatsu: My studies were exclusively with cotton which was glandless.

M. L. Rollins: The gossypol pigment gland is a very complicated structure. It has been isolated and studied. The first report was by Boatner and is in the Bailey book on cottonseed. In the Journal of American Oil Chemists last year or the year before, there are electron microscope pictures and light microscope pictures of the morphology of the gossypol gland, and we have reprints of these papers at the Laboratory.

Harold Corey: Have industry people considered separation techniques for protein and other constituents of seeds based upon the concept that these things come in neat, little packages? What is the status of this work?

A. M. Altschul: You are referring no doubt to Dr. Dieckert's work in which he used the non aqueous system and was able to grind up peanuts and separate the protein bodies from the cell wall material, from the nuclei and from other constituents. Since then, we have been able to do a similar thing in an aqueous system. We have been able to grind up peanuts in sucrose solutions under such conditions that the protein bodies, such as Dr. Yatsu showed, did not rupture. And we were able to purify those protein bodies and extract the protein from them. That is why we know that most of the protein that is in the peanut, for example, is in these protein bodies which can be isolated; and I would venture that this is a general principle. The late Dr. Morton showed the same thing for the bodies of immature wheat grains and Varner showed the same thing for protein bodies in the peas.

COTTONSEED PROTEINS

by
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Our previous speakers have pointed out certain aspects of the structure of the cells of cottonseed.

Each of the structures of the cell---the nucleus---the surrounding cytoplasm with its system of membranes embedded with specific organelles and an aqueous gelation of proteins, carbohydrates and inorganic ions--each of these structures or particulates of the cell are part of the storehouse and source of the proteins of cottonseed.

It was also noted that the cell of the hypocotyl or root axis (Figure 1) differs from that of the cotyledon (Figure 2) in that the cytoplasm of the cell of the cotyledon is completely filled with organelles which have been termed "protein bodies."

This difference in cell structure is reflected in the amino acid content of these two components of the seed. (Figure 3). Though the overall patterns are of similar proportions, the hypocotyl, which represents 5 to 10% of the seed is significantly higher in certain very important amino acids--namely lysine, threonine isoleucine and leucine.

This difference in the cell contents of the hypocotyl and cotyledon can also be demonstrated by the water extraction of these two components of the seed.

If the cells of the hypocotyl and cotyledon are lysed by mechanical rupture in the presence of water, centrifuged at 14, 000Xg. and lyophilized, the following results are obtained. (Figure 4). Fifty percent of the hypocotyl and 37% of the cotyledon are extracted by weight. This material includes free amino acids, peptides, carbohydrates, proteins and the lipids of the fatty layer which floats on the surface. This represents 40% of the total nitrogen of the hypocotyl but only 23% of the nitrogen of the cotyledon. When the fractions are dialyzed, a much larger percent of the weight and nitrogen of the material extracted from the cotyledon is lost. This would suggest that a greater proportion of the water-soluble material of the cotyledon is composed of free amino acids, peptides and carbohydrates. There also seems to be a greater proportion of acetone soluble or lipid-like material in this fraction of the cotyledon.

The seeds used in this experiment were the glandless variety. They were allowed to equilibrate intact, with water, overnight at 4 °C., dissected by hand into the cotyledon and hypocotyl components minus the residual indosperm layer and extracted with diethyl ether for 5 days at 4°C. This served to both dehydrate and remove the extractable lipid.

If one compares the amino acid content of the water soluble fraction of the hypocotyl with that of the whole hypocotyl, (Figure 5) it is obvious that there is a concentration of lysine in the water soluble fraction. Threonine is present in higher proportion than serine and glycine, alanine and phenylalanine are all present in higher concentration. All others are present in lesser amounts with arginine showing the greatest deficiency.

The same comparison (Figure 6) of the cotyledon and its water soluble fraction shows an even greater difference between the two as might be expected since this represents only 23% of the nitrogen. The

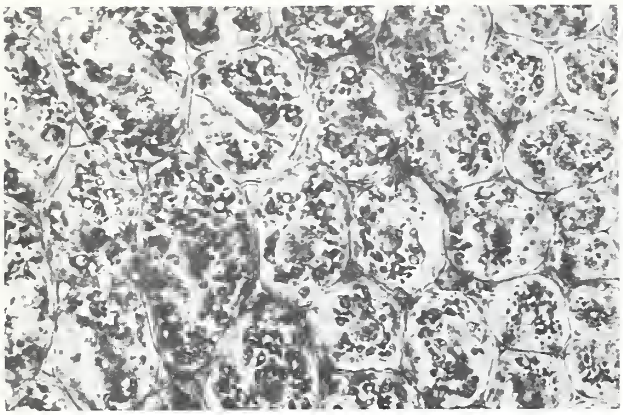


Figure 1.--A cross-section in the lower half of the Cottonseed Hypocotyl of freeze-dried, paraffin embedded material stained with Chlorozol B viewed under phase at a magnification of 1100

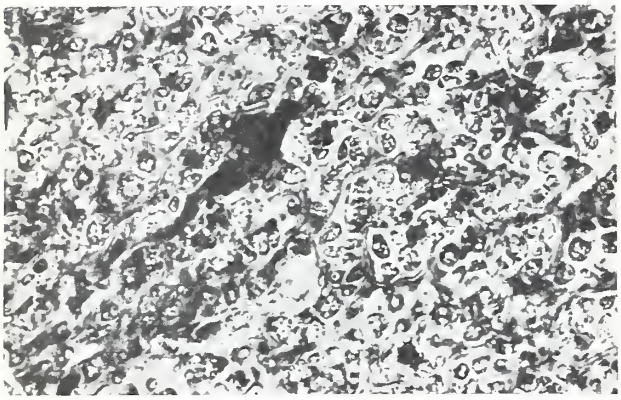


Figure 2.--A cross-section of a cottonseed cotyledon freeze-dried, paraffin embedded, stained with Chlorozol B viewed under phase at a magnification of 1100

AMINO ACID CONTENT OF COTTONSEED HYPOCOTYL AND COTYLEDON g/16g. N

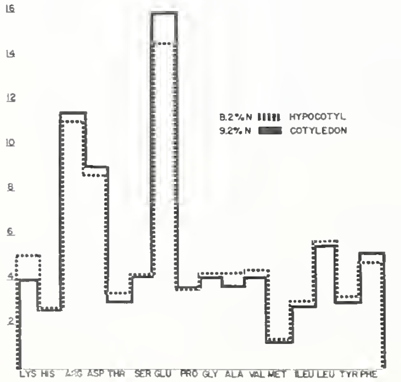
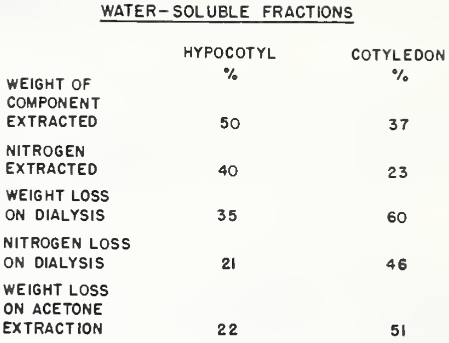


Figure 3.



WATER-SOLUBLE FRACTIONS

	HYPOCOTYL %	COTYLEDON %
WEIGHT OF COMPONENT EXTRACTED	50	37
NITROGEN EXTRACTED	40	23
WEIGHT LOSS ON DIALYSIS	35	60
NITROGEN LOSS ON DIALYSIS	21	46
WEIGHT LOSS ON ACETONE EXTRACTION	22	51

Figure 4.

AMINO ACID CONTENT OF HYPOCOTYL AND H_2O SOLUBLE FRACTIONS g/16g. N

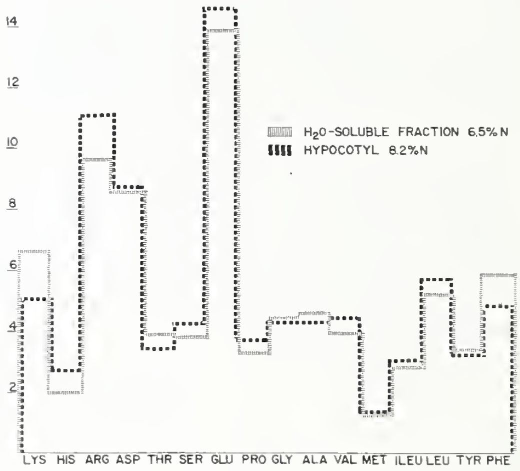


Figure 5.

AMINO ACID CONTENT OF COTYLEDON AND H_2O SOLUBLE FRACTIONS g/16g. N

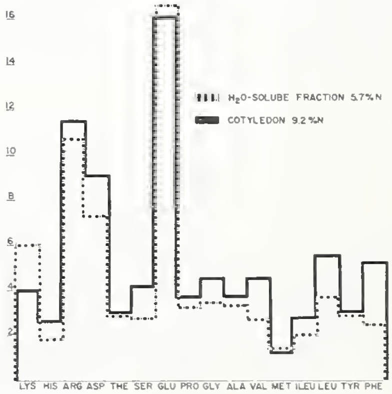


Figure 6.

water soluble fraction again shows a high concentration of lysine as well as an increase in glutamic acid and methionine. All other amino acids are present in distinctly lower concentration, particularly the neutral and aromatic amino acids.

A comparison of the amino acid content of the water soluble fractions (Figure 7) shows obvious differences. However, the magnitude of these differences is not reflected in a comparison of the electrophoretic patterns of the proteins of these fractions seen in the upper right corner. The pattern to the left is that of the hypocotyl fraction, the other that of the cotyledon fraction. These patterns were obtained by gel electrophoresis (1) in Tris-EDTA-Boric Acid Buffer at pH 8.7 (2). What appears to be insoluble material at the point of origin is due to the collodial suspension of the lipid components of the fractions which were otherwise completely soluble in the buffer.

This lack of a major difference in the patterns may be due, in part, to free amino acids or peptides which would not stain with nigrosine. Some difference in amino acid content, however, can be attributed to the relative proportion of the proteins present. In the middle grouping of two bands of the hypocotyl, the upper band is present in much lower concentration and reverse proportion to the same grouping in the cotyledon fraction. There also appears to be a band in the hypocotyl fraction which is not present in the cotyledon fraction. Aside from these differences, the striking similarity in the protein patterns of these fractions does concur with the idea that these fractions represent the soluble proteins of the cytoplasm. It should be pointed out that in these electrophoretic patterns the relative intensity of a particular band of one component may be compared with what is assumed to be the same band in a second component; however, the intensity of staining from one band to another cannot be equated with protein content since the staining ability depends upon amino acid configuration and not protein concentration.

If the water extracted residue of each component is further extracted with a solvent designed to dissociate and solubilize nucleoproteins--specifically 1.5 M salt in 50% Ethanol (3)--a fraction of each is obtained which is characteristically high in arginine and glutamic acid and low in lysine. (Figure 8).

Though the amino acid patterns have similar profiles, the differences seen may be accounted for by the relative intensities of the protein bands present in the fractions. The cotyledon fraction on the left has four predominate bands of differing intensities, one of which does not appear in the hypocotyl fraction whose six bands differ in intensity from those in the cotyledon.

The high electrophoretic mobilities and the sedimentation characteristics of these proteins suggest that these are basic low molecular weight proteins which is in keeping with present ideas concerning nucleoproteins.

If one examines the cotyledon from the aspect of the particulates present, one may note in the light microscope that the protein bodies appear to consist of two structures--small globoids or spheres embedded in an oval to round matrice. These spheres have been characterized by Yatsu and Altschul (4) as particles high in lipid and low in protein which do not sediment below a force of 12,000 X g. A seemingly pure fraction of these spheres isolated by density gradient centrifugation of the water extracted, homogenized cotyledon shows four components on electrophoresis and an amino acid pattern which is very low in lysine (Figure 9) and has a notably different ratio of arginine and glutamic acid to the other amino acids. This fraction has the highest content of glucosamine and glalactosamine noted in any fraction. Also there is a ratio of almost one between the μ moles of ammonia and the μ moles of glutamic and aspartic acid--a fact which is not observed in any other fraction.

A solvent composed of Tris-EDTA-Boric Acid buffer pH 8.7 and .5 M salt can be observed under the microscope to completely dissolve the protein bodies. Such a fraction contains the same four bands associated with the spheres as well as a band which has the same mobility as that of the first band of the alcohol salt or nucleoproteins.

The amino acid pattern of this material which is 15.7 percent N, is extremely high in glutamic acid. It is also high in arginine, valine, and phenylalanine, but again low in lysine. It may be noted that the alcohol-salt fraction which does not contain the broad slow moving band has an amino acid pattern not too dissimilar from that of the alkali-salt fraction. It would seem that the amino acid pattern of these fractions are greatly influenced by the amount of this particular salt-sensitive band which coprecipitates with the other proteins.

Indeed, if one examines the amino acid patterns of the water-extracted residue or the 0.5 M or 1 M salt extracts of the cotyledon, (Figure 10) they all show essentially the same characteristics of low lysine and high arginine and glutamic acid.

The slightly higher lysine content of the water-extracted residue may be due to the presence of a protein fraction (Figure 11) which seems to be associated with the wall of the cell and represents only a small portion of the total nitrogen. This pattern is most unusual in that the lysine and the neutral amino acid content are high aspartic acid and leucine are present in almost equal proportion and are greater than arginine. In turn the glutamic acid content is relatively low.

Figure 12 is a qualitative electrophoretic picture of the proteins of the cotyledon. The water soluble or cytoplasmic constituents seen in column five consist of 8 to 14 proteins which are high in lysine and cystine and are associated, perhaps only by solubility, with carbohydrates. This lysine is therefore readily available for binding and/or destruction. Also any extraction or isolation procedure which removes these proteins would yield protein low in lysine.

The proteins of column four are the alcohol-salt or nucleoproteins which are also extracted by 0.5 M or 1 M salt solutions along with the broad slow moving band of the protein bodies, as seen in column two. The two forward moving bands of the spheres and protein bodies (columns one and three) seem to be absent in straight salt extracts. However, the protein of any salt extract (acidic or alkaline) of the water extracted cotyledon does precipitate as uniform spheres, a fact noted by Ritthausen (5) in the first isolation of cottonseed globulin.

Columns one and three are those proteins associated with the spheres and whole protein bodies. The proteins of the spheres are not only low in lysine, but contain little or no cystine. The proteins of the whole protein bodies are characterized by low lysine and high arginine and glutamic acid and do contain cystine. Which of these bands is associated with the spheres, which the protein matrix, and which the membranous network that seems to outline or delineate the protein bodies remains to be elucidated.

I would like to thank Dr. Mann and Miss Carter of our laboratory without whom the amino acid analyses would not have been possible; Dr. Frampton for giving me the opportunity and freedom to work on this problem, and acknowledge the work of Dr. Altschul and his group on the nature of the particulates of cottonseed.

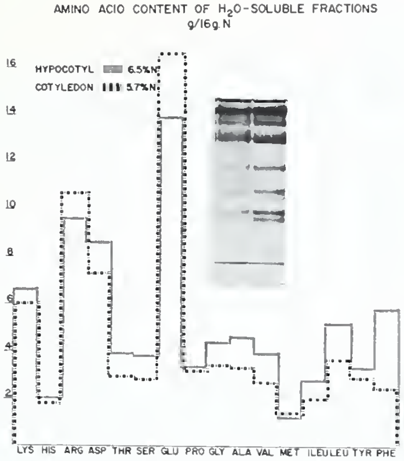


Figure 7.

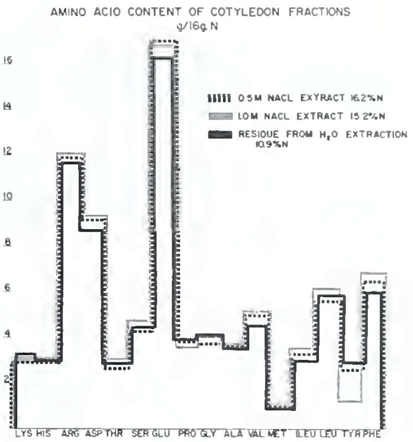


Figure 10.

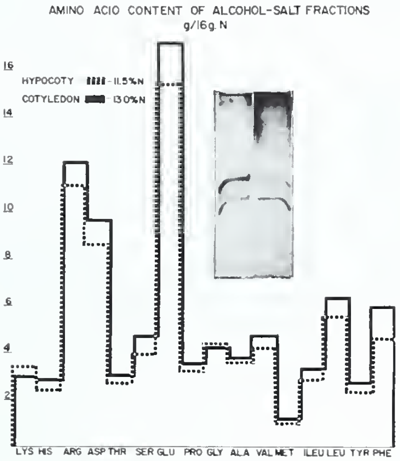


Figure 8.

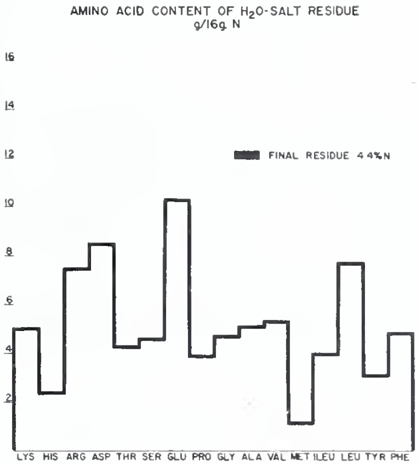
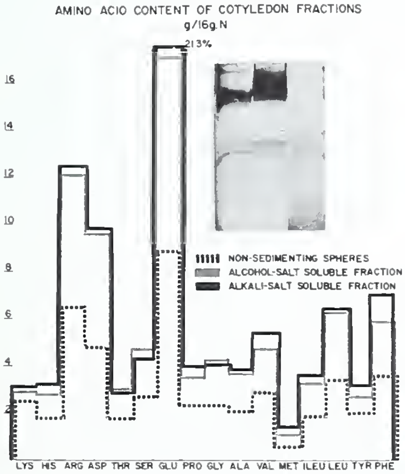


Figure 11.



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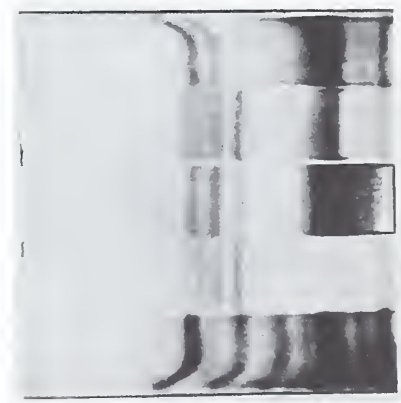

Figure 12.--Electrophoresis of cottonseed proteins

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DISCUSSION

M. L. Anson: We are open for discussion.

A. M. Altschul: I would like to point out a lesson to be learned from the work presented by Mrs. Martinez. It has always been interesting to note that lysine in the soybean is not easy to destroy and that it survives moderate processing; it takes rough treatment to lower the lysine in the soybean, whereas the lysine in the cottonseed is very easily rendered unavailable. Probably part of that easiness of destruction in the cottonseed comes from the gossypol, but I don't think that is the entire story. I think more of the story comes from the information that Mrs. Martinez presented.

When we talk about the lysine content of a soybean as being 5% or 6%, we are talking about an average, when we talk about the lysine content of the cottonseed being 4.3%, we are talking about an average; but as she pointed out, there are certain proteins that have a lot of lysine and certain proteins that have very little lysine. How they are distributed--in packages or not, or in relationship to materials that can react with them, such as carbohydrates--determines their sensitivity to processing. I think that as more of the information she is getting develops and more information is known about the proteins, we will be able in a more sensible way to understand and perhaps control the destruction of lysine and perhaps other amino acids during processing.

THE ROLE OF GOSSYPOL

by
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The presence of gossypol in cottonseed protein concentrates presents two separate and distinct problems. The first of these is that the presence of high levels of free gossypol will cause unfavorable physiological effects which under certain circumstances may become serious. The second factor is that the chemical reaction between gossypol and protein results in a reduction in the availability of lysine and hence a reduction in protein quality.

Feeding trials for the purpose of nutritional evaluation should be so designed as to measure one factor or the other and not both at the same time. Unfortunately this has not always been done. The result has been that many experiments throughout the years have been grossly misinterpreted.

With respect to the first factor namely free gossypol, it is not at all difficult to reduce the level of free gossypol to safe levels by the control of processing variables. The trick is to do this without unduly reducing protein quality. This is not so easy. The job becomes more and more difficult with increasing levels of gossypol in the raw seed.

What happens to ingested gossypol? Recent studies in our laboratory in which radioactive gossypol (formyl C^{14} gossypol) was fed to chicks give some insight into this problem. It seems likely that similar patterns will be found in other species.

Approximately 3.5 mg of radioactive gossypol was given in gelatin capsules on each of four consecutive days. The total activity of the four doses was approximately 1μc. The feces were collected on the day following the administration of each capsule and on one additional day, totaling five collections for each chicken. The animals were sacrificed on the sixth day and the organs removed for the determination of C^{14} activity.

The first slide (Table I) shows average values obtained with 5 chicks on a practical diet. C^{14} activity appeared in all of the tissues tested with the highest concentration in the liver.

The next slide (Table II) shows that only a part of the C^{14} activity found in the tissues was soluble in ethyl ether indicating that part of the gossypol was probably bound to protein.

The next slide (Table III) shows that essentially all of the administered activity was recovered either in the tissues or in the feces. It is evident that only a small fraction of ingested gossypol is absorbed from the digestive tract.

A number of types of feeding experiments have shown that quantitatively the harmful effects of gossypol can be reduced and sometimes eliminated altogether by increasing the amount of good quality protein in the diet. In order to obtain further information on this relationship we took a practical type ration as a control and mixed the same ration half and half with fish meal and fed the two rations to chickens. Radioactive gossypol was administered to both groups and the C^{14} activity determined in the tissues as before. The significance of the data given in Table IV is that fish meal reduced the amount of gossypol deposited in the kidney, muscle, liver and blood. These are the tissues which account for most of the absorbed gossypol.

TABLE I

C^{14} ACTIVITY IN TISSUES OF CHICKS FED FORMYL-C^{14}-GOSSYPOL

Values expressed as mμ c/g fresh tissue

Practical Diet

Tissue	Chick No.					Average
	8	9	12	13	14	
Heart	0.344	0.197	0.132	0.167	0.220	0.212
Brain	0.062	0.025	0.164	0.187	0.171	0.122
Lung	0.308	0.151	0.280	0.387	0.267	0.279
Spleen	0.404	0.187	0.275	0.175	0.288	0.266
Kidney	0.302	0.465	0.541	0.443	0.510	0.452
Muscle	0.181	0.036	0.139	0.263	0.167	0.157
Liver	2.637	2.240	5.104	3.620	7.050	4.130
Blood			0.265	0.300	0.496	0.354

Throughout the years there have been persistent reports, notably by Ed Eagle, that quantitatively the toxicity of isolated cottonseed pigment glands could not be accounted for by the gossypol content of the glands. Recently we investigated this subject in our laboratory and confirmed the previous work. The glands were then fractionated and a new pigment was isolated. We named the new pigment gossyverdurin because of its green color and its structural relationship to gossypol.

Fig. 1 shows the separation of gossypol from gossyverdurin on a silicic acid column. Table V shows a comparison of the toxicity of gossyverdurin, gossypol, and other known gossypol related pigments. The LD-50 values were determined by standard procedures. Gossyverdurin like gossypol reacts with para anisidine. Consequently, if we analyze gossyverdurin for free gossypol by standard procedures, it appears to have about 25% free gossypol. This of course is not the case.

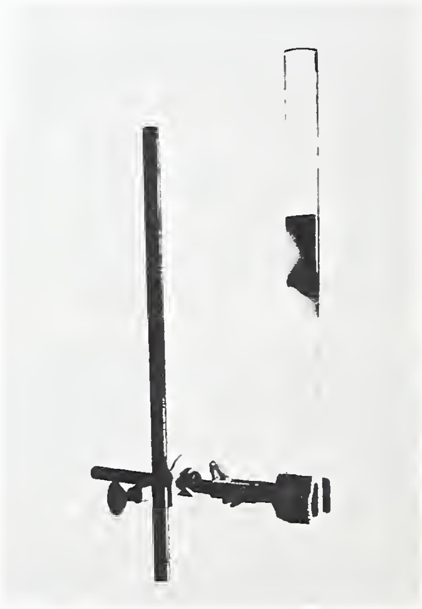


Figure 1
The separation of gossypol and gossyverdurin on a silicic acid column.

TABLE II

TOTAL AND ETHYL ETHER SOLUBLE C^{14} ACTIVITY IN TISSUES FROM CHICKS FED FORMYL-C^{14}-GOSSYPOL

Practical Diet

Tissue	Total radioactivity		Ethyl ether soluble activity	
	mμ c/g	Activity recovered* %	mμ c/g	Activity recovered* %
Heart	0.212	0.079	0.017	0.007
Brain	0.122	0.028	0.026	0.002
Lung	0.279	0.105	0.022	0.015
Spleen	0.266	0.017	0.051	0.005
Kidney	0.452	0.259	0.031	0.023
Muscle	0.157	3.079	0.011	0.086
Liver	4.130	4.377	0.394	0.732
Blood	0.354	1.590		
Total		9.53		

*Expressed as % of administered activity found in tissues

TABLE III

RECOVERY OF C^{14} ACTIVITY IN TISSUES AND FECES FROM CHICKS FED FORMYL C^{14}-LABELED GOSSYPOL

Values expressed as % of administered formyl-C^{14}-gossypol

Practical Diet

Chick no.	Recovered in tissues	Recovered in feces	Total activity recovered
	%	%	%
8*	8.06	91.77	99.8
9*	3.64	97.50	101.1
12	9.60	85.01	94.6
13	11.87	83.00	94.9
14	11.31	89.04	100.4
Average	8.90	89.26	98.2

*Blood samples were not analyzed.

TABLE IV

EFFECT OF FISH MEAL ON THE DEPOSITION OF GOSSYPOL IN CHICK TISSUES AS DETERMINED BY MEASUREMENT OF C^{14} ACTIVITY AFTER ADMINISTRATION OF FORMYL-C^{14}-GOSSYPOL

	mμ c/g Fresh tissue		% Administered gossypol found in tissue	
	Practical diet	Fish meal	Practical diet	Fish meal
Kidney	0.45	0.33	0.26	0.23
Muscle	0.16	0.09	3.08	1.42
Liver	4.13	2.28	4.38	3.04
Blood	0.35	0.29	1.59	1.16

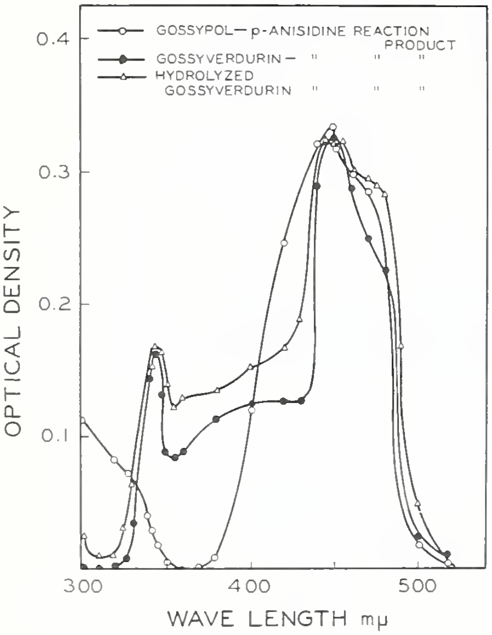


Figure 2
Absorption spectra of gossypol and gossyverdurin para-anisidine reaction products.

The absorption spectra of the para-anisidine reaction products are given in Figure 2. In addition to the absorption maximum at 447 mμ given by both gossypol and gossyverdurin, gossyverdurin shows a second maximum at 342 mμ.

TABLE V

TOXICITY OF GOSSYPOL DERIVATIVES

	LD-50 g/kg. body weight
Gossypol	2.565
Diamino gossypol	3.274
Gossypurpurin	6.684
Gossyverdurin*	0.659

* A newly discovered green pigment.

C. M. Lyman, A. S. El-Nockrashy and J. W. Dollahite, J. Amer. Oil Chem. Soc., 40, 571-575 (1963)

A. S. El-Nockrashy, C. M. Lyman and J. W. Dollahite, J. Amer. Oil Chem. Soc., 40, 14-17 (1963)

Gossyverdurin like gossypol reacts with the epsilon amino groups of lysine in proteins. The reaction appears to be more rapid than is the case with gossypol.

The question has been raised many times concerning the possible effects of small amounts of free gossypol in cottonseed protein preparations when they are used as human food. It is well to remember that such products are cooked after being combined with other food materials. Under such circumstances, free gossypol disappears rapidly. Table VI shows the rapid rate of reduction of free gossypol when a cottonseed protein preparation was simply boiled with water.

TABLE VI

EFFECT OF BOILING HEXANE EXTRACTED COTTONSEED MEATS WITH WATER

Boiled min.	Free Gossypol %	Total Gossypol %
0	1.45	1.63
5	0.12	1.67
10	0.09	1.63
15	0.07	1.62

Although somewhat irrelevant to our subject this morning, it will perhaps be of interest to this group that the Texas Station has now developed a safe high producing swine ration using 10% of solvent extracted cottonseed meal. Meals may be used which contain as much as 0.2% of free gossypol. Protein quality in solvent extracted meals is typically high and the way is now clear for the effective use of such meals in commercial swine production. The ill effects of gossypol are completely blocked by the use of low levels of iron salts in combination with small amounts of fish meal.

Gossypol and Protein Quality

It is now well known that gossypol adversely affects protein quality by reacting with the epsilon amino group of lysine forming a compound which cannot be utilized for building body tissue. To express this effect of gossypol in another way we may say that gossypol reduces lysine availability. Before proteins can be absorbed and utilized, they must be hydrolyzed by the proteolytic enzymes of the digestive tract. One of the most inportant of these is trypsin. Table VII shows the effect of blocking the epsilon amino group of lysine with gossypol on the liberation of the individual amino acids in invitro tests. The free amino acids were determined in the digest by the use of an automatic amino acid analyzer. As expected, the amount of lysine liberated was less in the sample treated with gossypol. Of particular significance is the fact that not only lysine liberation but also the liberation of other amino acids was reduced, some even more than lysine, as for example glutamic and aspartic acids. These data do not necessarily mean that gossypol reacts with other amino acids besides gossypol. It is well known that basic groups such as the epsilon

TABLE VII

THE EFFECT OF BLOCKING LYSINE EPSILON AMINO GROUPS WITH GOSSYPOL ON THE LIBERATION OF AMINO ACIDS BY TRYPTIC DIGESTION

The figures represent the % reduction in amino acid liberation when the bound gossypol was increased from 0.32% to 86%

Amino Acids	Period of Digestion 8 hrs.	24 hrs.
Lysine	13.99%	16.10%
Histidine	22.22%	19.10%
Arginine	+4.28%	2.54%
Aspartic Acid	36.45%	61.46%
Threonine	28.48%	39.08%
Serine	28.24%	32.96%
Glutamic Acid	42.22%	49.21%
Proline	-----	43.89%
Glycine	24.76%	41.71%
Alanine	25.98%	32.90%
Valine	20.54%	33.80%
Methionine	9.17%	26.86%
Isoleucine	12.24%	27.20%
Leucine	+4.22%	13.58%
Tyrosine	+2.19%	17.00%
Phenylalanine	+8.22%	13.86%

amino group of lysine are essential for the action of trypsin. When we block this group with gossypol, we interfere with the utilization of the protein as a whole with some parts of the protein being affected more seriously than others.

While studying this same problem from another point of view we were quite surprised to find that the enzymatic digestion of cottonseed flours and also protein-gossypol complex preparations, apparently released free gossypol from bound gossypol. The same results were obtained by the use of a variety of different proteolytic enzymes (Table VIII). The determination of free gossypol was carried out by standard analytical procedures. However, when we attempted to demonstrate the presence of free gossypol by chromatographic tests, no free gossypol was found but rather gossypol peptide complexes which analyzed as free gossypol.

Continuation of this investigation demonstrated that any form of bound gossypol which is soluble in the reagents used in the analytical procedure will give a positive value for free gossypol. These findings do not invalidate the use of the standard free gossypol method on cottonseed flours and meals. They do mean that partially digested materials, however prepared, will likely give erroneous results. To date we have not found an enzyme which will split the gossypol protein linkage to the epsilon group of lysine.

The general conclusion to be drawn with respect to the relationship of gossypol to protein quality is that any amount of bound gossypol in a cottonseed protein preparation will have some adverse effect, the magnitude depending on the amount of gossypol present. Ideally, then, we should get rid of gossypol completely by whatever means is the most economical. From a practical standpoint it is well to remember that very useful products can be made which contain moderate levels of bound gossypol. A lot of difficulties and disappointments can be avoided if we attempt to do this with standard processing methods and equipment only in areas where the seed is typically low in total gossypol (less than 1 percent of the raw kernels).

TABLE VIII

APPARENT LIBERATION OF BOUND GOSSYPOL BY PROTEOLYTIC ENZYMES

Enzyme	Type of sample	Bound gossypol in sample	Free gossypol before hydrolysis	Apparent free gossypol after hydrolysis
Pancreatic extract	Cottonseed meal extracted with acetone	0.98	0.00	0.04
Crystalline trypsin	" " "	0.98	0.00	0.03
Crystalline α chymotrypsin	" " "	0.98	0.00	0.04
Pancreatic extract	Serum albumin-gossypol complex	2.97	0.00	1.73
Crystalline trypsin	" " "	2.97	0.00	1.79
Crystalline α chymotrypsin	" " "	2.97	0.00	1.63

DISCUSSION

R. M. Leverton: Dr. Lyman's paper is open for discussion, please?

D. A. Shirley: I would like to ask Dr. Lyman if he has any information which would allow him to propose a possible structure for gossyverdurin.

C. M. Lyman: This is actively being investigated at the present time. It would appear that it has carbonyl groups similar to gossypol which react with amino groups such as we have in proteins. The infrared absorption spectra (and we don't know what to make of this) would indicate the absence of phenolic stretching bands. Possibly these are combined in some way. Other than this, I think it is just speculation. I hope that at the next meeting we will have something more to report.

We can say this, that the compound after it has been isolated can be combined with protein and removed again. The p-anisidine compound obtained from the protein is not the same as that obtained with gossypol. We can say that gossyverdurin contains about 1% of nitrogen which is not in the form of an amino acid because hydrolysis gives amonia instead of an amino acid. Now, if we take a lysine gossypol complex and hydrolyze it, lysine is liberated. So, it isn't an amino acid. The structure of this nitrogen linkage is intriguing.

D. A. Shirley: What lines of evidence do you have that your material is a pure and homogeneous substance?

C. M. Lyman: Gossyverdurin is very unstable and as the isolation proceeds some decomposition takes place. So it is a difficult thing to get it absolutely pure. The only lines of evidence that we have are that spectrographically on columns and on paper, it moves as a single green band. I think we are going to have to make a stable derivative and work on this.

R. M. Leverton: Are there other questions?

A. M. Altschul: Carl, you showed that when you cooked cottonseed in water you decreased the free gossypol content very markedly. Do you have any parallel information on the nutritive value of such products? Were they damaged in the process?

C. M. Lyman: Yes, I wanted to limit the number of slides. When we boiled cottonseed meal in water, nitrogen solubility went way down. The free gossypol also decreased markedly. However, the reduction in nutritive value was very, very slight except in cases where large amounts of free gossypol were converted to bound gossypol. In such cases the lysine with free epsilon amino groups decreased and a corresponding reduction in nutritional value occurred.

FACTORS AFFECTING THE NUTRITIVE QUALITIES OF COTTONSEED

by
Vernon L. Frampton
Southern Utilization Research and Development Division
New Orleans, Louisiana

There is a wide variability in the nutritional quality of cottonseed meals for nonruminants. We, at the Southern Utilization Research and Development Division, have undertaken to account for this variability on the basis of cottonseed meal composition, and to account for it in a quantitative manner. In essence, we undertook to develop a quantitative definition of cottonseed meal in terms of its constituents that will have the highest attainable nutritive quality for nonruminant animals. This was undertaken to set up guidelines for the practical production of cottonseed meals of the highest attainable quality.

It didn't occur to us at the outset that we could produce cottonseed meals with protein quality as good as toasted soybean meal protein, but the feeding tests that have been conducted with the meals produced recently by my group at the Southern Division indicate that our meals are as good as or better than toasted soybean meals in promoting growth and are equal or higher in their protein efficiency ratios than are toasted soybean meals. We feel very confident that we will, as we learn more about the factors that affect the nutritive quality of cottonseed meals, produce meals that are superior to the meals we produce at the present time.

I am sure that there are present some who are not completely at home with multiple regression analyses and analyses of covariance that are used in processing quantitative biological data. Perhaps you will bear with me if I outline briefly the philosophy that forms the basis of the conclusions we have reached in this paper. Most of us have the desire in our research to hold all but two variables constant, and note the relationship between the two selected variables. Our training in the physical sciences teaches us to think this way. The type of research where only two variables are considered is possible in the classical physical sciences where the total number of variables one has to deal with is limited to three. One has only two independent variables to contend with in classical physical science. The basic fundamental relationship between variables in classical physical sciences is given by the equation

$$du = \left(\frac{\delta u}{\delta x}\right)_y dx + \left(\frac{\delta u}{\delta y}\right)_x dy$$

where the subscript y denotes that the variable y is constant and the subscript x denotes that the variable x is constant. The three variables u, x, and y may be pressure, temperature, and volume, or they may be heat capacity, index of refraction and tensile strength, or any set of three variables one may wish to choose. The major part of research in classical physical science is that of determining the function within either or both parentheses in the equation.

No such elegant relationship exists in quantitative biology. In biology there are many independent variables, and it is extremely difficult to design an experiment in biology where only one independent and only one dependent variable are encountered. Methods for working up data where more than one independent variable is encountered have developed, and our alternative in quantitative biology is to rely on these methods for determining quantitative relationship between variables.

In our experiments to determine and quantitively assess the factors that influence the nutritive quality of cottonseed meals we deliberately selected meals that varied widely among themselves in many chemical characteristics. In some instances we made the conscious effort to induce maximum variability in as many variables as possible. All we wished to know about the meals was the intensity value for each variable. Thus we obtained meals from mills that we knew produced poor meals and we obtained meals from mills that we knew produced good meals. We prepared very good meals and very bad meals in the laboratory to obtain even wider ranges of quality than can be realized in meals purchased on the open market. These many diverse meals were fed to our experimental animals, and we ended up with a scramble of data (about 20,000 animals, about 100 different rations and many cottonseed meals) that appeared to be in hopeless chaos. Growth responses, protein efficiency ratios, total lysine, available lysine, gossypol, energy levels, etc., all varied considerably.

It is possible to bring order out of an apparently hopelessly chaotic mass of data of this type. If, for example, we plot the weights of chicks from an experiment against the weights observed for the chicks on various cottonseed meal-containing rations against the lysine content of the meals, we obtain a scatter diagram of the type that is shown in Figure 1. Now, by Gauss' method of least squares we can determine the curve that will best fit the data where each datum is given equal weight. We are assuming, as a first approximation, that the relationship between growth response and lysine is linear. The curve shown in Figure 2 is referred to normally as the regression curve.

In Figure 3, I have indicated the average value for weight gain and lysine on the regression curve, and there are included two data for weight gain. The perpendicular is dropped from each point on the graph to the ordinate for the average of mean-weight-mean-lysine-content, as shown in the figure. Each perpendicular is divided into two portions, as shown, and one portion is designated as "d" and one as "D". Now the sum of either all of the "d"s or all of the "D"s

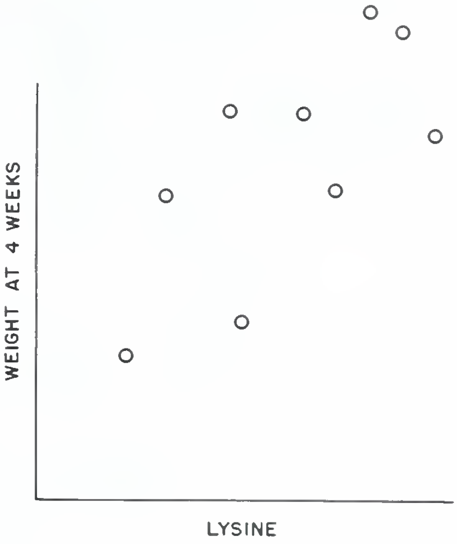


Figure 1

Scatter diagram of weights of chicks at 4 weeks plotted against lysine contents of cottonseed meals.

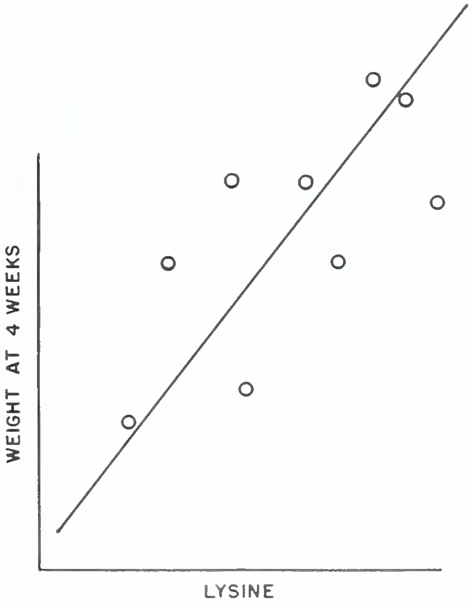


Figure 2

Relation of weights of chicks at 4 weeks on lysine of cottonseed meals.

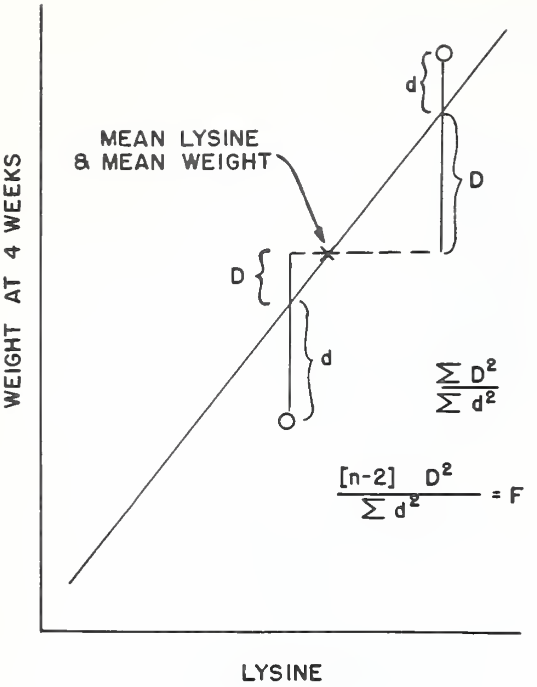

MEAN LYSINE
& MEAN WEIGHT
WEIGHT AT 4 WEEKS
d
D
D
d
$\frac{\sum D^2}{\sum d^2}$
$\frac{[n-2]\ D^2}{\sum d^2} = F$
LYSINE

Figure 3

Illustration of calculation of F ratio with one independent variable.

must equal to zero, since the regression curve is the best fit for all of the data. However, neither the sum of the squares of the "d"s, nor the sum of squares of the "D"s, is zero, and we can use the ratio of the sum of the squares of the "D"s to that of the sum of the "d"s as a measure of the degree of fit between the experimental data and the regression curve. The greater the ratio, the better the fit, and when the ratio is infinitely large the fit is perfect and the coefficient of correlation is 1. 0. If the ratio is zero there is no semblance of a fit and the coefficient of correlation is zero. In actual practice, we multiply the ratio by $\left(\frac{N - 2}{1}\right)$ where N is the total number of observations made. We will not go into a discussion of the reason for this coefficient at this time. The quantity (N - 2) is referred to as the number of degrees of freedom. The quantity $\frac{\Sigma D^2}{\Sigma d^2}\ \frac{N - 2}{1}$ is called the F ratio.

It is permissible to assume that some other independent variable in addition to lysine is also influencing the rate of growth of the chicks in an experiment where several different cottonseed meals are fed. This additional factor may be the energy content of the ration, for example. In such a situation we plot our data in a space defined by the coordinates of weight gain, lysine content of the rations and the energy content of the rations. Again linear relationships are assumed, as a first approximation, and under these circumstances we have to deal with a regression plane rather than a regression curve. It is possible, by the method of least squares, to determine the plane that fits the data best, and it is possible to calculate the F ratio as a measure of the degree of agreement between the data and the regression plane, Figure 4. Here we multiply the ratio by $\left(\frac{N - 3}{2}\right)$. We have, in this instance, one degree less of freedom, namely (N - 3).

Should it happen that the energy is not relevant, the ratio calculated for the two independent variables will be smaller than that calculated from the lysine data alone. Should the ratio be larger, we are justified in concluding that energy is relevant to growth of the chicks. We can assess the relative importance of the two independent variables to the growth response and we can also calculate the degree of confidence we can place on the relative importance of each.

The geometric representations were included for illustration purposes. Actually we do not need the geometric representations, and we are not limited in the analyses to only two independent variables. The problem is an algebra problem and we may consider as many dimensions, or variables, as our degrees of freedom will permit.

We examined, as the independent variables in our comprehensive feeding tests, lysine, available lysine, and all of the amino acids of cottonseed proteins, with the exception of tryptophane. Included among the 27

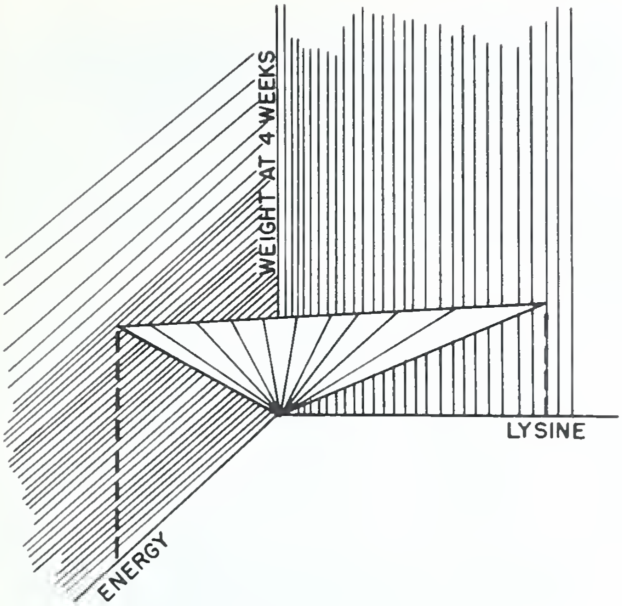


Figure 4

Illustration of calculation of F ratio with two independent variables.

independent variables studied were total energy of the rations, energy contributed by cottonseed meal, residual lipids in the meals, degree of saturation of the residual lipids, ratio of phospholipids in residual lipids to total residual lipids, inorganic phosphate, nonlipid organic phosphate, free gossypol, total gossypol, total sugars, reducing sugars, crude fiber, residual lipids, intensity of Halphen reaction shown by residual lipids, etc. I calculated the coefficients of correlations for every possible simple correlation. Only those variables where some degree of significance can be attached to the coefficients of correlation between growth and the individual variables were selected for further study. It is recognized that simple correlations can be misleading and that there is an element of danger in this restriction in eliminating some variables from consideration because the simple correlations are small. It was felt, however, that variables whose contribution to the total variance in the nutritive quality of cottonseed meals may be masked in these preliminary experiments might be identified later when the contribution of the several factors to growth response of young animals is better understood. I found correlations of varying degree of significance between growth rate and total lysine, available lysine, total gossypol of cottonseed meals and crude fiber, histidine and arginine of rations which contained cottonseed meals. Some correlations obtained in a broiler experiment are shown in Table I.

F ratios calculated from this experiment are tabulated in Table II. Note that the F ratio for total lysine is reasonably large, and it is much larger than that for total gossypol. The F ratios for the other variables are materially smaller than those for either lysine or gossypol. Attention is called to the fact that the F ratio for free gossypol is virtually zero. Evidently the total variance in growth is accounted for more completely by the variance in lysine than by the variance of any other constituent.

A study of the combined effects of the cottonseed meal constituents, taken two at a time, indicated that the F ratio is insignificantly small unless the total lysine is included as an independent variable; therefore only results of the computations where lysine was included are reported here. In every instance where lysine and some other variables were considered, the F ratio is less than it is for lysine alone. This statement is true for computations where total and free gossypol were considered. It is concluded that any contributions these two variables, namely free and total gossypol, may make to the total variance in growth are completely masked by the influence of lysine.

This is further verified by the data shown in Table III where the effect of total gossypol is determined after allowing for the contributions of lysine. Note that the F ratio for the contribution of total gossypol is extremely small.

The study was extended to the consideration of independent variables, taken three at a time, in an effort to detect the masked contributions they might make to the total variance in growth. The F ratios calculated in all instances were insignificantly small unless total lysine was included as one of the independent variables. However, in every instance, the F ratio calculated for the three independent variables was less, and significantly so, than that calculated for lysine alone. Typical data are reported in Table II; no masked effects were detected.

TABLE I. --Coefficients of Correlation Between Weights of Broilers at 4 Weeks and Constituents of Cottonseed Meals or of Rations Fed

Feeding test	Total lysine	Arginine	Histidine	Free Gossypol	Total Gossypol	Crude Fiber
1	0.92	0.56	-0.11	-0.19	-0.68	0.28
2	0.82	0.63	0.35	-0.17	-0.69	0.33
3	0.87	0.60	0.30	0.06	-0.77	0.35
4	0.90	0.59	0.34	0.20	-0.71	0.29

TABLE II. --F Ratios for Contributions of Cottonseed Constituents to Total Variance in Growth of Broilers

Constituent	F ratio
Total lysine	70
Total gossypol	3.5
Free gossypol	0.1
Arginine	2.7
Crude fiber	0.2
Lysine and total gossypol	23
Lysine and free gossypol	30
Lysine and crude fiber	26
Lysine and arginine	21
Lysine, total gossypol and arginine	16
Lysine, total gossypol and crude fiber	12

TABLE III. --F Ratios for Contributions of Cottonseed Constituent on the Total Variance of Broilers

Experiment	Constituent	F ratio
A	Effect of total gossypol after allowing for effects of total lysine	0.2
B	ditto	3.4
C	ditto	2.8

This same conclusion was reached when the study was extended to a consideration of five independent variables.

The number to be subtracted from the total number of observations, to give us the degrees of freedom in a multiple regression study increases by one with each added dimension. Therefore, the calculations of F ratios, in experiments with several independent variables, are limited by the size of the experiment, e.g., the number of meals or rations fed. Thus when the number of variables considered, in any one analysis equals the number of meals or rations fed, we have to multiply our ratio $\left(\frac{\Sigma D^2}{\Sigma d^2}\right)$, by zero. It is possible, however irrespective of the number of degrees of freedom to determine the equation that relates the rate of growth of young animals to the independent variables. An example for four independent variables is shown in the equation:

$$W = K + (1570 \pm 750)L + (37 \pm 47)C - (98 \pm 206)G - (107 \pm 165)A$$

Where W = predicted weight at 4 weeks, K = constant, L = lysine, C = crude fiber, G = total gossypol and A = arginine. We can, through the use of matrix algebra, determine the standard deviation for each coefficient in equations of the type given. This is given for each coefficient in the equation. For example, the standard deviation for the lysine coefficient is ± 750 and that for crude fiber is ± 47.

It is difficult to assess the confidence levels that may be assigned to the several coefficients when small numbers of meals or rations are fed (eight different meals were compared in the example cited), but considering that the standard deviations determined here are larger than the calculated coefficients for crude fiber, total gossypol and arginine, there is no reason for assuming that the coefficients calculated from the growth response data for broilers differ from zero.

It is interesting to note that the prediction that the variance in total gossypol contents of cottonseed meals used in broiler rations contributes very little to the total variance in growth is verified by the data reported in Table IV. The rations were designed to produce good growth response, and the meals were the acetone-hexane-water mixed solvent meals with high levels of available lysine, and with varying amounts of total gossypol. Data are for weights of broilers at 4 weeks. The simple coefficient of correlation between growth response and available lysine is insignificantly small - so is the coefficient between growth response and total gossypol. The coefficient of correlation calculated between growth response and total gossypol is 0. 2, and the odds for significance are nil.

The evidence is that we can account more completely for the total variance in growth observed from feeding a number of meals or rations by the variance in total lysine than we can by the variance of any other constituent or combination of constituents studied. Our data indicate, however, that the variation in nutritive quality of commercial cottonseed meals studies is not accounted for completely by the variation in the available lysine level in meals, vide supra. There are some presently unidentified and unmeasured factors that also contribute to the variability in growth that are observed when several cottonseed meals are fed.

TABLE IV. --Growth of Broilers on Acetone-Hexane-Water Meals

Meal	Wt. of Broilers (grams)	Available Lysine G/16 g. of N.	Total Gossypol %
A	335	4. 15	0. 38
B	401	4. 05	0. 21
C	384	4. 10	0. 32
D	427	4. 26	0. 16
E	328	4. 26	0. 30
F	423	4. 25	0. 31
G	386	4. 25	0. 25
H	396	4. 28	0. 22
I	371	4. 22	0. 47
J	362	4. 29	0. 29
K	381	4. 30	0. 22
L	380	4. 24	0. 33

Our data indicate that the variance in protein efficiency ratio bears the same general relationship to the cottonseed meal constituents as does the rate of growth. Gossypol data are reported in Table V. Note that the F ratio for lysine is significantly larger than for the total gossypol or free gossypol and that the F ratio calculated from combination of lysine and total gossypol is less than that calculated for lysine alone. The F ratio for total gossypol, after allowing for lysine is very small. In general, prediction of the protein efficiency ratio based on the lysine content of the meal will agree more closely with the observed value than will the predicted ratio based on any of the other variables. Moreover, a prediction of the protein efficiency ratio based on lysine alone will agree better with the observed ratio than will the protein efficiency ratio calculated from combinations of lysine and the other variables. The evidence from all of our broiler data is that for broilers on rations

TABLE V--F Ratios for Contributions of Cottonseed Constituents to Variance in Protein Efficiency

Constituent	F ratio
Total lysine	8
Total gossypol	3
Free gossypol	0.03
Total gossypol and lysine	3.4
Total gossypol after allowing for total lysine	0.3

where cottonseed meal serves as the protein supplement, the effect of lysine on the total variance in protein efficiency ratio is so great that the contributions (if any) to the total variance of the other factors studied (including free and total gossypol) are completely masked.

Reference in the above experiments has been limited for the most part to the total lysine. By total lysine, I mean the lysine as determined by the ion-exchange procedure described by Martinez and Frampton (1). Attention was also given to the lysine in cottonseed meals with epsilon amino groups free. Lysine in cottonseed proteins that has the epsilon amino group free is referred to as available lysine, for reasons that are developed in the course of this discussion.

It has been demonstrated repeatedly (1) that lysine in cottonseed meal protein is destroyed when the meals are heated. In addition, it has been shown that epsilon amino groups of some of the lysine in pure proteins are bound when gossypol is added to a solution of the proteins. That is, some of the epsilon amino groups of the lysine in pure proteins that have been treated with gossypol are no longer free for reaction with 2, 4-dinitrofluorobenzene (2) as shown in Table VI.

TABLE VI--Reaction of Gossypol with Free Epsilon Amino Groups of Lysine in Proteins

Protein	Available lysine (Grams/16 grams of N)	
	Original protein	Gossypol treated protein
Bovine serum albumin	11.9	8.3
Beta-lactoglobulin	12.5	10.2
Egg albumin	6.7	4.1
Cottonseed globulin	3.5	3.0

The effect of gossypol on cottonseed proteins was also investigated. Meal (CM-71), prepared from raw glandless cottonseed flakes by thorough extraction with commercial hexane at ambient temperatures, served as a source of native cottonseed proteins that had never been exposed to reaction with gossypol. This meal (Table VII) served as source material for a study of the relative effects of gossypol and of raffinose (the major simple carbohydrate present in cottonseed) on the binding and on the destruction of lysine and on the nutritive quality of the meal proteins as determined through repletion of protein depleted rats (3). The meal was thoroughly extracted with 80 percent aqueous ethanol--the extraction was continued until the extraction of sugars was complete. The resulting material was designated meal CM-71-A. Raffinose, in an amount equivalent to that occuring in cottonseed meal, was added to an aliquot of the alcohol extracted meal (CM-71-A) to produce CM-71-A-R. Gossypol, equivalent to 1 percent of the total meal, was added to a second aliquot to produce CM-71-A-G. Both gossypol and raffinose were added to a third aliquot to produce CM-71-A-R-G. Each aliquot was then divided into three portions, one of which was fed without further treatment, one portion was heated in an autoclave at 105° C. for 20 minutes, while another portion was heated to the same temperature for 60 minutes. These various preparations were then fed to protein depleted rats in tests where casein served as the reference protein.

TABLE VII--Effects of Treatment on Properties of Glandless Cottonseed Meal Proteins

Meal	Heated (Mins.)	Nutritive index	Total lysine G/16 g. of N.	Available lysine G/16 g. of N.
71	0	101	4.2	4.1
	20	76	3.7	3.7
	60	68	3.5	3.1
71-A	0	96	4.4	4.4
	20	101	4.3	4.3
	60	86	4.2	4.1
71-A-R	0	93	4.4	4.4
	20	86	4.3	4.2
	60	71	4.0	3.6
71-A-G	0	90	4.4	4.2
	20	75	4.4	3.9
	60	61	4.3	3.5
71-A-R-G	20	85	4.2	4.1
	60	70	4.1	3.5

Data for the nutritive index (the ratio of gain of rats on the experimental protein to that of rats on casein) and for total and epsilon amino free (available) lysine are included in Table VII. Note that substantial destruction of lysine occurred when CM-71 was heated for 60 minutes and that the destruction under comparable heating conditions was substantially less when the alcohol soluble materials were first removed from the defatted meal. The addition of either raffinose or gossypol had no marked effect on the destruction of lysine during the autoclaving of CM-71-A-R or CM-71-A-G for 60 minutes. Binding of lysine in CM-71-A was insignificant under the condition of heating. A comparison of the available lysine data for meals CM-71, CM-71-A, CM-71-A-R, CM-71-A-R-G lead to the conclusion that substances in cottonseed meal, in addition to raffinose and gossypol and which are extracted with 80% ethanol, have the property of both destroying and binding lysine when hexane extracted cottonseed meal is heated.

It is now recognized from our current work that the treatment of cottonseed meal with chemically pure gossypol is not completely realistic in trying to simulate the reactions that occur between gossypol and other cottonseed constituents when cottonseed flakes are cooked for processing purposes. The new evidence is that gossypol does not occur in cottonseed in the free state, and gossypol derivatives obtained from the pigment glands may react differently than does pure gossypol.

The data reported in Table VII were examined to ascertain if bound lysine can be used by the protein depleted rats. Analyses of the regression of reduction of nutritional index on lysine destroyed and lysine bound is shown in Table VIII.

TABLE VIII.--Analyses of Variance of Regression of Reduction of Nutritive Index (glandless cottonseed meal) on Lysine Destroyed and Lysine Bound

Source of Variance	Sum of squares	Degrees of freedom	Mean Square	F ratio	Probability points
Regression on lysine destroyed					
Due to regression	370	1	370	2.6	N.S.
Residual	1,718	12	143		
Total	2,088	13			
Regression on lysine destroyed and lysine bound					
Due to regression	1,529	2	765	15	.01
Residual	559	11	51		
Total	2,088	13			

Note that the F ratio for bound lysine is large in comparison with that for lysine destroyed. Apparently the protein depleted rat, i.e., the rat that is starving for protein, is unable to use lysine unless the epsilon amino groups are free. Thus only that lysine with free epsilon amino groups is biologically available to the rat, and it is referred to hereafter as available lysine.

Comparable observations were made with chicks fed cottonseed meals. Weights at two weeks of chicks on a suboptimum protein diet are reported in Table IX, along with the total and available lysine data (4). Analysis of the regressions to determine the effect on growth is shown in Table X. Note that the F ratio for available lysine is large in comparison with that of total lysine. Note also that the F ratio for available and bound lysine is less than that for available lysine, and that the F ratio for bound lysine, after allowing for available lysine, is zero. It is concluded that bound lysine does not contribute to the growth of broilers.

Similar results were obtained with swine. Shoats cannot use lysine unless the epsilon amino groups are free.

It was implied earlier in this discussion that there is a wide variability in the available lysine in commercial cottonseed meals. The degree of variability may be noted from the data included in Table XI, where typical analyses are reported. Note that the level of available lysine in commercial meals ranges from 2.22 grams to 3.75 grams per 16 grams of meal nitrogen. Analyses of variance of these data show that although there is a wide variability in available lysine among the hexane extracted and prepress solvent meals, the differences are not attributable to the method of processing. Analyses of the data show, however, a decreased level of lysine in the screw press meals, as compared with hexane extracted or prepress solvent meals. This is attributable to the screw press operation; screw pressing does more damage to the protein quality of cottonseed meals than do either hexane or prepress solvent extraction. It is obvious from the data, even without statistical analysis, that the level of available lysine is significantly higher in the meals prepared with the acetone-hexane-water solvent mixture than they are in the commercial meals.

These A-H-W meals and their preparation will be the subject of my discussion tomorrow.

TABLE IX. --Chick Growth and Available Lysine in Cottonseed Meals

Meal	Weight at 2 weeks	Lysine (grams/16 grams of meal N)		
		Total	Available	Bound
A	290.3	3.7	3.7	0.0
B	206.3	3.8	3.0	0.8
C	165.7	3.6	2.5	1.1
D	265.0	4.0	3.4	0.6
E	180.7	3.6	2.9	0.7
F	224.4	4.0	3.1	0.9
G	249.0	4.2	3.5	0.7
H	116.0	2.9	1.6	1.3

TABLE X. --Analyses of Variance of Regressions of Weight of Chicks on Forms of Lysine in Cottonseed Meal

Form of lysine	Source of variance	Sum of Squares	Degrees of freedom	Mean Square	F ratio	Probability points
Total lysine	Due to regression	12,944.7	1	12,944.7	6.1	0.05
	Residual	12,707.1	6	2,117.6		
	Total	25,651.8	7			
Available lysine	Due to regression	20,369.0	1	20,369.0	23.1	0.01
	Residual	5,282.8	6	880.5		
	Total	25,651.8	7			
Bound lysine	Due to regression	6,180.6	1	6,180.6	1.9	N.S.
	Residual	19,471.2	6	3,245.2		
	Total	25,651.8	7			
Available and bound lysine	Due to regression	20,369.0	2	10,184.5	9.6	0.25
	Residual	5,282.8	5	1,065.5		
	Total	25,651.8	7			
	Due to bound lysine after allowing for available lysine	0.0	1	0.0	0.	N.S.
	Available lysine	20,369.0	1	20,369.0	7.7	0.025
	Residual	5,202.8	5	2,641.4		
	Total	25,651.8	7			

TABLE XI. -- Available Lysine in Cottonseed Meals Prepared by Several Methods

	Screw press	Prepress solvent	Hexane extraction	Acetone-Hexane-Water azeotrope extraction
	2.53	3.14	3.30	4.23
	3.07	3.21	3.31	4.18
	2.70	3.38	3.20	4.15
	3.22	3.70	2.82	4.18
	3.42	3.60	3.64	4.26
	2.22	3.17	3.75	4.25
	3.21	3.36	3.12	4.25
	3.11	3.36	3.67	4.28
Average	2.84	3.35	3.39	4.22

We made the effort to reverse the chemical reactions that cause binding of epsilon amino groups in cottonseed meal protein to gain a better insight into the binding of both gossypol and lysine. There was some success enjoyed from these efforts (5). It was assumed that the epsilon amino groups are bound in the form of Shiff bases. Accordingly the meals were treated with primary amines to drive the reaction in the reverse direction and liberate epsilon amino groups of lysine from the Shiff bases. Increased levels of available lysine and decreased levels of total gossypol were obtained. Typical data are included in Table XII. Note that the reduction of total gossypol obtained on treating a solvent damp marc prepress solvent extraction was spectacular --the level dropped from 1.25 percent in the marc (air dried basis) to 0.03 percent for the amine treated material. Evidently it is possible, by proper treatment, to reduce the total gossypol in cottonseed meals to virtually zero.

The evidence is that varying degrees of lysine destruction and binding in cottonseed meals is responsible to a very large degree for the varying nutritive quality of commercial meals. Optimum nutritive quality may be realized only if the lysine in the seed proteins is retained in the native form.

How about the other amino acids? The complete amino acid profiles have been determined for many meals. Profiles are shown in Table XIII for two meals that produced extremes in growth in a swine feeding experiment. One gave the best growth and the other the poorest. The average daily gain in weight of the shoats was 0.37 for meal 113 and 1.6 for meal 106. The only differences that appear in the amino acid profiles of these two meals that are greater than the analytical error are the total lysine and the available lysine. Some essential amino acids, other than available lysine, may not be present in cottonseed meals at optimum concentrations. However, such amino acids cannot contribute to the total variance in growth because the variance of such amino acids is zero. Our data do not tell us if some other amino acid is limiting in cottonseed meals.

TABLE XII. --Liberation of Bound Lysine by Amines (Solvent Damp Prepress Marc)

Treatment	Total Gossypol	Available lysine
Solvent damp marc	1.36	3.4
Stand 16 hrs. in octylamine and extracted with $CHCl_3$	0.03	3.7
Reflux 30 min. 10% 3-aminopropanol in MeOH	0.21	3.9

The implication from the remarks offered earlier is that gossypol may be innocuous in broiler rations. It is not desired to give this impression. While we have not been able to measure the contribution of total gossypol to the total variance in growth of broilers or protein depleted rats, the data obtained with swine indicate that the contribution of the variance of total gossypol to the total variance in growth of swine is about 1/20 that of the contribution of the variance of available lysine.

There are physiologically active derivatives of gossypol in cottonseed meals that are not measured by the total gossypol methods. Actually, the total gossypol methods do not measure total gossypol, but rather they measure that gossypol that may be liberated from derivatives in cottonseed meal on mild acid hydrolysis, or which may be displaced from combination in the meals by an amine. That these methods do not measure the physiologically active forms of gossypol in cottonseed meals is demonstrated by the data plotted in Figure 5. The yolks of stored shell eggs produced by hens that have received a ration containing cottonseed meals and oil are discolored--they are of a dirty greenish brown color. The chromogen appearing in the yolks has not been identified, but it is well established that it is caused by feeding gossypol to laying hens. We have plotted in Figure 5 the concentration of the chromogen, as determined by Frampton, _et_ _al_. (_6_) against the free and total gossypol contents respectively of the meals fed. Chromogen concentrations were determined after storage periods of 3, 6, and 9 months. Note a complete lack of correspondence between the concentrations of the chromogen in the yolks and either the free or total gossypol concentrations in the meals fed--the simple coefficients of correlation are virtually zero. We know that the pigment arises because of gossypol in the rations, but the concentration of the pigment is not related to either the total gossypol or free gossypol in the meals, as measured by the current methods. No chromogen occurs in the yolks of eggs produced by hens on cottonseed meals with very low gossypol contents. At the present time we have no method for determining the concentration of physiologically active gossypol in cottonseed meals.

The implications from the discussions up to this point are that a major portion of the total variance in growth of broilers and shoats and in protein repletion of protein depleted rats receiving cottonseed meals as the protein supplements is accounted for by the variance in available lysine. Our evidence is that other constituents studied do not contribute measurably to the total variance in growth, with the exception of the small contribution total gossypol makes to the variance in the weight gain of shoats. It was established that a portion of the lysine in cottonseed meals is destroyed when the meals or meats are heated and that a por-

TABLE XIII. --Amino Acid Composition of Cottonseed Protein of Two Cottonseed Meals

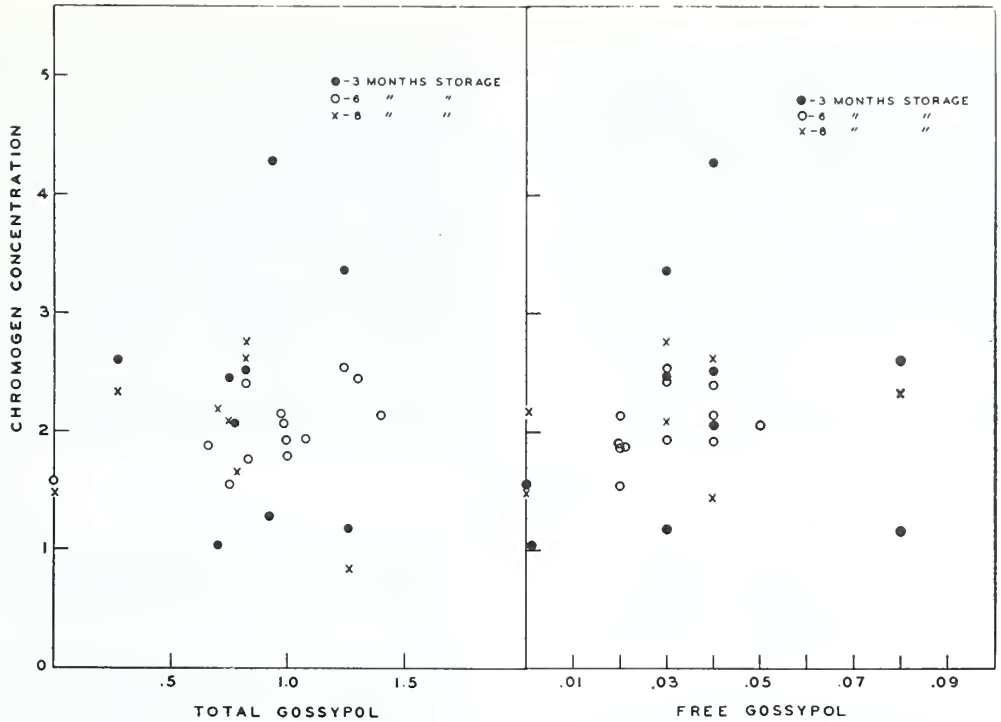
	106	113
Total lysine	3.7	2.9
Available lysine	3.3	1.3
Arginine	11.9	8.2
Histidine	2.5	2.3
Aspartic acid	9.1	8.2
Threonine	3.1	2.9
Serine	4.2	3.9
Glutamic acid	18.0	18.8
Proline	3.8	3.6
Glycine	4.2	3.6
Alanine	3.9	3.8
Valine	4.4	4.3
Methionine	1.5	1.3
Isoleucine	3.1	2.9
Leucine	5.7	5.5
Tyrosine	3.1	2.8
Phenylalanine	5.3	5.0

tion that escapes destruction is bound in such a way that the epsilon amino groups are not free to react with 2, -4 dinitrofluoro benzene. Such bound lysine is not available to broilers, shoats or to protein depleted rats. It was also established that gossypol is bound in cottonseed meal when the flakes are cooked for oil extraction, and there is evidence that some form of physiologically active gossypol is present in cottonseed meals.

The evidence is that lysine is not destroyed or bound and that the gossypol is not bound in the meals if cooking operations are avoided. In order to produce meals of highest nutritional quality, it is necessary to maintain the native lysine and to eliminate, insofar as possible, all of the gossypol from the meal. Two of the guidelines for the best possible cottonseed meal are: 100 percent native lysine and zero percent gossypol in the meals. These two objectives may be reached if the cooking step in cottonseed processing is avoided and if gossypol is extracted before it has the opportunity to become bound in the meal.

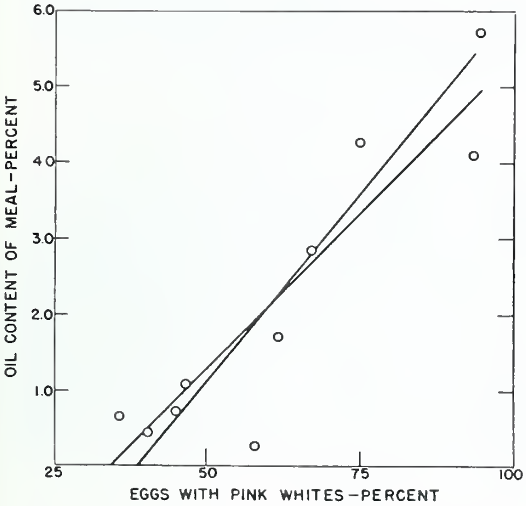
There is an additional factor that must be considered if we are to produce cottonseed meals of the highest nutritional quality and which are not suspect. This is the factor present in the residual oil of cottonseed meals that induces pink discoloration in the whites of stored shell eggs that are produced by hens fed cottonseed meal. It is suspected that the responsible factor is cyclopropenoid fatty acid -malvalic acid-that is present in cottonseed oil (crude) to the extent of about 1 percent. The relationship between the appearance of pink whites in eggs produced by hens receiving cottonseed meals and the residual oil content of the meals is shown in the two regression curves of Figure 6. Here the percentage of eggs with pink whites appearing after six months of storage is plotted against the percent residual oil in the meals. Zero percent eggs with pink whites are obtained only when the oil content of the meals is low. Evidently the residual oil in cottonseed meals must be reduced to the lowest level possible if meals that are not suspect are to be produced. This means that virtually quantitative extraction of oil from raw cottonseed flakes must be achieved. Zero residual oil in the meals is the third guide line for the production of the best possible meals.

There is yet another important factor, one which has not been identified and about which very little is said. This factor must be very seriously considered in any effort



Relation of average relative chromogen concentration in yolks to gossypol of meals

Figure 5



Occurrence of pink whites of stored shell eggs and oil content of meals fed in laying rations

Figure 6

to produce the ideal cottonseed meal. I refer to the lethal factor that is responsible for unexplained mortalities that occur among swine receiving certain cottonseed meals.

We do not know why some cottonseed meals kill pigs or why some cottonseed meals do not. We have noted 100 percent deaths caused by cottonseed meal in some of our experiments. This statement is not presented to create alarm--actually meals that kill pigs were selected for our experiment as a part of our research to identify the cottonseed meal constituents that are responsible for the deaths. The identification of their lethal factor and its elimination from cottonseed meals is now our major research job. It is encouraging that meals that most satisfactorily meet the guidelines set up above (namely the acetone-hexane-water meals) have not killed any pigs.

There seems to be a heat labile antinutritional factor in cottonseed meals. It may be noted, for example, in Table VI that there was an enhancement in the nutritive index when CM-71-A was heated for 20 minutes. Additional data bearing on this subject will be reported later by Professor A. B. Watts.

One may contend, in summary, that the definition of the ideal cottonseed meal will contain the statements: 100 percent of the original seed lysine is native in the meal; residual gossypol and gossypol derivatives in the meal are zero; residual crude oil in the meal is zero. These three guidelines may be approached if the cottonseed meats or flakes are not cooked in the processing of cottonseed for oil, and if the oil and gossypol derivatives of the seed are extracted rapidly and virtually quantitatively. These guidelines are closely approached if the raw moist flakes are extracted with the hexane-acetone-water azeotrope (7).

The definition of the ideal cottonseed meal suggested above is not complete since it does not contain a statement about the factor present in many meals that is lethal to swine. Also, the definition cannot be complete until the total variance in growth of young animals and in the protein efficiency ratio has been completely accounted for by the variance in cottonseed meal constituents. Our research is directed toward the solution of these two problems.

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DISCUSSION

L. H. Bean: May I ask you, it is not particularly important, but I am curious about the method of correlation that you use. In the first slide you showed us a diagram, and in the second one you showed us the same diagram with the straight line drawn through it using a method that you said goes back a hundred years. I gather from that, that you use entirely linear correlations, straight line relationships. That is question number one.

And question number two, how do you deal with these multiple situations where you have more than two factors involved?

R. M. Leverton: I think this would be helpful, but we cannot get into an argument about statistics. Can you answer in no more than 12 sentences?

V. L. Frampton: We use the linear relationship. I think in reality, the curves are not linear. They are probably S shaped. But the data we have are too few in number to determine what the shapes of the surface are, and as a first approximation we used the linear procedure. Now in the question of multiple variables, where we were dealing with curvilinear relationships, I think it would be better if I saw you privately and talked to you about that.

L. H. Bean: Well, may I make this one observation. The reason for my asking this question is that I don't think it is necessary to go back a hundred years for a method of dealing with the net relation of one variable to another in the presence of other variables. There is a method published in 1929, known as the Bean Graphic method of multiple curvilinear correlation for exactly this type of problem where a straight line doesn't really represent the basic facts. The Graphic method permits dealing with the other variables present when you want to isolate a particular one.

V. L. Frampton: We followed a procedure of getting first approximations because we were interested in determining which factors are important nutritionally as a guide to us in the selection of processes for production of cottonseed meals of high quality.

L. H. Bean: It is quite possible that your F ratios may give you a wrong impression of relative importance.

ROLE OF CYCLOPROPENOID FATTY ACIDS IN UTILIZATION OF COTTONSEED FLOUR AND MEAL

by
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Introduction

In recent years cyclopropenoid fatty acids have been regarded by some people as undesirable components of cottonseed products and a considerable research effort has been directed toward removal or inactivation of these materials. It is quite possible that these fatty acids may one day find a new and beneficial role just as the element selenium has discarded the stigma of toxicity and joined the list of desirable and essential elements.

Because there are by necessity gaps of knowledge in research, and because a scientist must at times resort to impure preparations and substitutes rather than pure materials, it is conceivable that erroneous conclusions may have been drawn from published reports. Therefore it seems essential that we review briefly together the evidence which implicated cyclopropenoid fatty acids as undesirable components of many plants in the botanical order Malvales. It seems logical to also explore the levels of materials tested to determine if these are realistic. Lastly, reference will be made to data presented by Michigan workers which seemingly negate the assumed interrelationship between Halphen test, cyclopropenoid fatty acids and biological manifestations of the acids.

History of Cyclopropenoid Fatty Acid Research

The Halphen test, a red color developed through use of carbon disulfide, has long been used to detect cottonseed oil (Halphen, 1897). It is now known that oils from most plants of the families which comprise the botanical order Malvales, including okra, cotton, kapok, hollyhock, several mallows, and Rose of Sharon, will yield a positive Halphen test (Phelps, 1962). Fatty tissue of animals which ingest some of these plant oils also yield a Halphen test (Lorenz et al., 1933; Lorenz and Almquist, 1934; Lorenz, 1939; Masson, 1957; Almquist et al., 1934; Shenstone and Vickery, 1959).

Almost ten years ago Gunstone (1955) stated, "An acid with a cyclopropene ring is present in small amounts in cottonseed oil and appears to be responsible for the Halphen test". Shenstone and Vickery (1956) confirmed that the material giving the Halphen test was in the saponifiable fraction of cottonseed oil. Employing a 19-carbon cyclopropenoid fatty acid of known structure (Nunn, 1952), Faure (1956) deduced by means of infrared spectral changes that the cyclopropenylene configuration of the fatty acids was essential for development of the Halphen test color.

MacFarlane et al. (1957) reported that malvalic acid gave a Halphen reaction and Craven and Jeffrey (1959) confirmed the material as an 18-carbon cyclopropenoid acid. This acid was later reported by the Australian workers to be the predominant cyclopropenoid fatty acid of cottonseed oil and to exist at a level of 0.7 to 1.5 percent in laboratory pressed crude cottonseed oil (Shenstone and Vickery, 1961). The same authors and other scientists (Earle et al., 1959) have estimated the level of total cyclopropenoid fatty acids in laboratory pressed crude cottonseed oil at 1 to 2 percent. Levels in commercial crude cottonseed oil have been found to vary between 0.57-0.79 percent (Kemmerer, 1963). Presumably the heat encountered during commercial oil extraction accounts for the reduced levels of cyclopropenoid acids compared with levels in laboratory pressed cottonseed oils.

Biological Changes Attributed to Cyclopropenoid Fatty Acids

A) Accelerated Pink White Discoloration of Eggs and Associated Disorders

A pink white discoloration of cold stored eggs has long been associated with ingestion of plant oils of the order Malvales (Sherwood, 1928, 1931; Almquist and Lorenz, 1933; Lorenz, et al., 1933; Lorenz and Almquist, 1934). The pink white discoloration, due to reaction of conalbumin with iron (Schaible and Bandemer, 1946b; Azari and Feeney, 1961), may occur in stored eggs from hens that have not ingested portions of or byproducts from plants of the order Malvales (Thompson, 1934; Schaible et al., 1946). Thus the responsible material in plant oils of the order Malvales appears to accelerate a change which may occur normally in eggs stored for an extended period of time.

Masson et al. (1957) showed that eggs from hens fed daily 100 mg of the 19-carbon cyclopropenoid fatty acid sterculic displayed pink discoloration after one month of cold storage. Similarly, Shenstone and Vickery (1959) found that partially purified malvalic acid would induce pink white discoloration in stored eggs.

Associated defects in the eggs from hens fed malvalic acid observed by Shenstone and Vickery (1959) included; (a) increased yolk pH, (b) yolk discoloration, (c) increased water content of yolks, (d) increased size and altered texture of yolks, and (e) increased concentration of iron and nonprotein nitrogen of egg whites. Feeding of crude cottonseed oil or cottonseed meal to hens has resulted in similar changes in yolk pH (Schaible et al., 1946; Frampton et al., 1962), salmon colored yolk discoloration and increased content of yolk water (Evans et al., 1954; 1959), altered texture of yolks (Sherwood, 1928), increased content of iron in egg whites (Schaible and Bandemer, 1946a), and increased content of nonprotein nitrogen in egg whites (Bandemer et al., 1946).

Therefore production of accelerated pink white discoloration of eggs and associated defects by feeding purified or partially purified cyclopropenoid fatty acids suggests that the materials in residual oil of cottonseed meal responsible for the same egg defects are also cyclopropenoid fatty acids.

B) Elevated Stearic and Depressed Oleic Acid

For many years increased levels of saturated fatty acids or increased hardness of animal fats has been associated with feeding of cottonseed products (Ellis et al., 1931; Deuel, 1955; Eckles and Palmer, 1916). Evans et al., (1960a, 1961) reported that fractions of crude cottonseed oil fatty acids soluble in acetone at -50 or -60°C. yielded positive Halphen tests, and induced pink white discoloration in stored eggs from hens fed the fractions. Furthermore, the eggs showed an elevation of stearic acid and depression of oleic acid proportional to strength of the Halphen test and degree of pink discoloration. Ingestion by the hens of *Sterculia foetida* seeds, a rich source of cyclopropenoid fatty acids, resulted in eggs with similar stearic and oleic fatty acid changes (Evans et al., 1961). Additional studies by the same group of workers revealed that a similar elevation of stearic acid and depression of oleic acid could be induced in liver, plasma, and ovary lipids of hens fed either of the two sources of cyclopropenoid fatty acids, crude cottonseed oil or *Sterculia foetida* oil (Evans et al., 1962a). The elevation of stearic acid in egg yolk lipids was not affected significantly by supplemental sources of oleic acid but refining the cottonseed oil reduced the magnitude of the phenomenon (Evans et al., 1963). The purified cyclopropenoid fatty acids, the previously mentioned acetone soluble cottonseed oil fatty acids, and *S. foetida* oil all yielded both a positive Halphen test and accelerated pink egg white discoloration. The acetone soluble fatty acid fraction of crude cottonseed oil, whole crude cottonseed oil, and *S. Foetida* seed all induced elevated stearic acid and decreased oleic acid in animal fats. Therefore, after putting all of these facts together it seems quite possible that the Halphen test positive fractions of the cottonseed oil apparently responsible for the elevation of stearic acid and depression of oleic acid were mainly cyclopropenoid fatty acids. Several additional reports of decreased iodine values or elevated stearic acid levels and depressed oleic acid following animal ingestion of sources of cyclopropenoid fatty acids strengthen the theory (Nordby, 1963; Schneider *et al*., 1962; Schneider, 1962b). It should be emphasized, however, that the writer is not aware of scientific data which confirms that ingestion of pure preparations of cyclopropenoid fatty acids induce elevated levels of stearic acid and depressed levels of oleic acids in animal fats.

C) Additional Disorders Associated with Ingestion of Cyclopropenoid Fatty Acids

Decreased egg production (Shenstone and Vickery, 1959), retardation of ovary, oviduct, and comb development and enlargement of liver and gall bladder (Schneider *et al*., 1962), increased chick embryo mortality (Schneider *et al*., 1961) and growth retardation of chicks (Schneider *et al*., 1962) and weanling rats (Schneider, 1962b) have been associated with ingestion of either sterculic acid or a rich source of the cyclopropenoid fatty acid, *Sterculia foetida* oil. From previously cited data it is obvious that the main cyclopropenoid fatty acid ingested in the above studies was of different chain length than the predominant cyclopropenoid fatty acid of cottonseed oil. More importantly the amount ingested exceeded amounts which might be encountered in practical rations if present methods for detection of cyclopropenoid fatty acids are reasonably accurate. Time does not permit a review of the methods but the following references will be found of interest (Deutschman and Closs, 1960; Shenstone and Vickery, 1956, 1961; Faure, 1956; Varma *et al*., 1957; Smith *et al*., 1960, 1961; Earle *et al*., 1960; Wilson *et al*., 1961; Faure and Smith 1956; MacFarlane et al., 1957; Magne *et al*., 1963; Harris *et al*., 1963). Bailey *et al*., (1961) have concluded that crude cottonseed oils give fictitiously high Halphen test responses so caution should be exerted in determining cyclopropenoid fatty concentration solely by the Halphen test. An even more important reason will be forthcoming.

Amount of Cyclopropenoid Fatty Acids Fed Experimentally in Comparison with Practical-Type Rations

A) Amount Required to Produce Accelerated Pink White Egg Discoloration

As mentioned previously, pink white discoloration has been shown to occur in stored eggs from hens fed rations devoid of known sources of cyclopropenoid fatty acids. The incidence of pink egg white production following ingestion of cottonseed meal or oil depends not only upon the amount of oil ingested but upon such factors as: (a) length of feeding period (Lorenz, 1939), (b) length of storage period (*ibid*), and (c) storage temperature (Shenstone and Vickery, 1959).

There are undoubtedly several other factors involved including concentration of cyclopropenoid fatty acids in the particular oil fed but lack of precise analytical techniques coupled with normal hen variation has prevented confirmation of this point.

It has been found that hens fed a ration containing the following levels of cottonseed oil will produce eggs which show pink white discoloration after storage at 2 °C. for the following lengths of time (Kemmerer *et al*., 1963):

TABLE I. --Effect of Ration Level of Cottonseed Oil on Pink Egg White Discoloration

Ration cottonseed oil %	Storage period in months when pink egg whites occurred (Stored at 2 °C)		
	1	3	6
2.0	+	+	+
.5	-	+	+
.2	-	-	+
.1	-	-	-

TABLE II. --Effect of Ingested Cyclopropenoid Fatty Acid-Containing Materials on Stearic: Oleic Acid Ratios

Ration level of Material fed %		Tissue analyzed	% Change in fatty acid from level induced by feeding basal ration		Reference
			Stearic	Oleic	
4	crude c/s* oil	hog back fat	+7.3	-9.7	Ellis *et al.*, 1931
8	" " " " " "	" " " " "	+9.3	-13.7	" " " " " " "
12	" " " " " "	" " " " "	+12.3	-17.5	" " " " " " "
2.5	" " " " " "	egg yolk lipids	+5.0**	-5.5**	Evans *et al.*, 1960a
0.7	crude c/s oil fatty acids soluble in acetone at -60°C	" " " " "	+5.3**	-6.2**	" " " " " " "
0.7	crude c/s oil fatty acids soluble in acetone at -50°C but precipitated at -60°C	" " " " "	+1.4**	-1.2**	" " " " " " "
2.5	crude c/s oil	egg yolk lipids	+11.8	-12.5	Evans *et al.*, 1961
0.17	*S. foetida* oil	" " " " "	+15.9	-19.7	" " " " " " "
10	c/w oil (presumably salad oil)	" " " " "	+3.8	-21.1	Wheeler *et al.*, 1959
2.5	crude c/s oil	" " " " "	+10.9	-14.6	Evans *et al.*, 1963
2.5	c/s salad oil	" " " " "	+5.1	-7.8	" " " " " " "
2.5	veg. oil shortening	" " " " "	+5.0	-4.7	" " " " " " "
2.5	crude c/s oil	" " " " "	+10.4	-17.0	" " " " " " "
2.5	crude c/s oil+7.5% corn oil	" " " " "	+10.2	-14.3	" " " " " " "
5	crude c/s oil +5% corn oil	" " " " "	+11.8	-16.4	" " " " " " "
10	crude c/s oil	" " " " "	+12.3	-21.4	" " " " " " "
2.5	crude c/s oil	" " " " "	+7.5	-10.5	" " " " " " "
2.5	crude c/s oil +7.5% olive oil	" " " " "	+6.6	-0.7	" " " " " " "
0.5	*S. foetida****	" " " " "	+8.9	-10.2	" " " " " " "
0.5	*S. foetida* seeds +10% olive oil***	" " " " "	+8.9	-5.5	" " " " " " "
2.5	crude c/s oil	hen plasma lipids	+4.5	-10.2	Evans *et al.*, 1962a
0.16	*S. foetida* oil	" " " " " "	+11.5	-14.1	" " " " " " "
2.5	crude c/s oil	hen liver lipids	+8.2	-8.5	" " " " " " "
0.16	*S. foetida* oil	" " " " " "	+8.4	-11.0	" " " " " " "
2.5	crude c/s oil	hen ovaries & contents	+11.0	-15.8	" " " " " " "
0.16	*S. foetida* oil	" " " " " "	+13.5	-19.4	" " " " " " "
2.5	crude c/s oil	hen heart lipids	+3.2	-1.6	" " " " " " "
0.16	*S. foetida* oil	" " " " " "	+3.9	-1.8	" " " " " " "
2.5	crude c/s oil	hen depot fats	+1.7	-2.3	" " " " " " "
0.16	*S. foetida* oil	" " " " " "	+3.4	-1.6	" " " " " " "
synthetic cyclopropenoid compounds equivalent to 25 mg *S. foetida* oil		egg yolk lipids	+16	----	Nordby, 1963

TABLE II. --Effect of Ingested Cyclopropenoid Fatty Acid-Containing Materials on Stearic: Oleic Acid Ratios (Continued)

* c/s - cottonseed

**% Above or below levels in eggs from hens fed basal ration plus cottonseed oil fatty acids in acetone precipitated at 2° C.

*** Amount of S. foetida seeds stipulated as -. 167% in another section of the same report.

Masson (1959) reported that hens fed for 12 days 2 to 4 mg/day of sterculic acid produced eggs which discolored pink when stored for three months. Similar results were reported by Nordby (1963).

B) Amount Required to Elevate Stearic Acid and Depress Oleic Acid

First we must assume that cyclopropenoid fatty acids alter stearic: oleic acid ratio since conclusive evidence appears to be lacking. The concentrations of materials shown in Table II have induced the noted changes in stearic and oleic acid.

The data cited in Table II show: (a) increased levels of stearic acid and decreased levels of oleic acid in fat of swine and in several hen tissues of animals fed rations presumed to contain cyclopropenoid fatty acids, (b) that increasing the ration concentration of crude cottonseed oil resulted in slight increases in animal fat levels of stearic acid and slight decreases in oleic acid, (c) the factor responsible for the fatty acid changes appears to be in the fraction of cottonseed oil fatty acids still soluble in acetone at -60°C., (d) the responsible factor appears to be reduced considerably in the manufacture of cottonseed salad oil from crude cottonseed oil, (e) some of the factors appear to be present in one brand of vegetable shortening, (f) *Sterculia foetida* oil or synthetic derivatives of the predominant cyclopropenoid fatty acid of the oil, sterculic acid, appear to exert a relatively strong effect on stearic: oleic acid ratios in animal fats, and (g) the induced depression of oleic acid levels in animal fats was not completely counteracted by supplementation with a source of oleic acid. Schnieder (1962a) reported similar observations relative to failure of oleic acid to completely counteract the effects of *S. foetida* oil.

All of the levels of crude cottonseed oil tested exceeded the amounts which would be encountered in use of cottonseed flour even if a child's diet was based solely on Incaparina.

C) Amount of Cyclopropenoid Fatty Acids Required to Induce Other Disorders Attributed to Cyclopropenoid Fatty Acids

1) *Decreased egg production*; Shenstone and Vickery (1959) reported that 25 mg/hen/day of either sterculic or malvalic acid did not significantly depress egg production but 250 mg/hen/day of sterculic acid caused cessation of laying within two days. The authors noted that this level of cyclopropenoid fatty acids was beyond the range attainable by incorporating common plant products into poultry rations. Based upon previously cited ranges of .57 to 0.79 percent cyclopropenoid fatty acids in crude cottonseed oil (Kemmerer, 1963) the following amounts could be calculated:

TABLE III. --Calculated Levels of Cyclopropenoid Fatty Acids Contributed by Following Amount of Cottonseed Flour

Grams cottonseed flour consumed daily	Residule fat in cottonseed flour		
	1%	2.5%	5%
30*	1.7- 2.4 mg	4.2- 5.9 mg	8.5- 11.9 mg

*Based on 75 g Incaparina containing 38% cottonseed flour.

Thus the highest calculated level of cyclopropenoid fatty acids would be less than half of the amount Shenstone and Vickery (1959) found to be without effect on egg production.

2) *Chick embryo mortality*: Schneider et al., (1961) found that 25 mg *S. foetida* oil oil/hen/day, increased chick embryo mortality above levels induced by feeding hens the basal ration. Mortality of chick embryos from hens fed 10 mg *S. foetida* oil daily did not differ significantly from mortality of embryos from hens fed the control ration. The level of cyclopropenoid fatty acids calculated to have been contributed

by the control and *S. foetida* oil rations in comparison with 30 grams daily cottonseed flour consumption are given in the following table.

TABLE IV.--Calculated levels of cyclopropenoid fatty acids contributed by *S. foetida* oil and cottonseed flour

Daily intake	Calculated level of cyclopropenoid fatty acids	Embryo mortality after 22 days incubation, %
25 mg *S. foetida* oil	10-18	82
10 mg " "	4-7	18
corn oil control	-	16
30 g c/s flour, 1% "fat"	1.7-2.4*	not tested
30 g c/s flour, 2.5% "fat"	4.2-5.9*	" "
30 g c/s flour, 5.0% "fat"	8.5-11.9*	" "

*Based upon .57 - .79% cyclopropenoid fatty acids in residual oil of flour

Naber and Morgan (1957) reported depression of chick hatchability after feeding 5 percent level of cottonseed oil but found that the depression could be overcome by vitamin supplementation. It is interesting to note that Schneider (1962b) partially counteracted deleterious effects of 5 percent *S. foetida* oil rations with B vitamins and that cyclopropane fatty acids share with certain long chain unsaturated fatty acids a biotin sparing action (Hofmann, 1962).

3) Retardation of ovary, oviduct, and comb development, enlargment of liver and gall bladder and growth retardation of chicks and weanling rats.

Studies in which the above changes were noted were confined to high levels of *S. foetida* oil which according to available evidence contributed a much greater concentration of cyclopropenoid fatty acids than would be contributed by a practical intake of cottonseed products. Furthermore available evidence indicates that the predominant fatty acid would be sterculic rather than malvalic acid.

The Enigma of the Michigan Reports

Several published (Evans *et al.*, 1962) and unpublished reports from Dr. R. J. Evans and his associates in Michigan have cast considerable doubt upon an apparently consistent relationship between (a) Halphen test, (b) pink white egg discoloration, and (c) the cyclopropenylene configuration of fatty acids. Dr. Evans and his associates found that a Halphen test positive fraction of crude cottonseed oil capable of inducing pink white discoloration of eggs could be divided into two fractions by urea clathration and crystallization from petroleum either at -60°C. In tabular form the two fractions had the following characteristics:

Fraction	Urea ppt	Halphen test	Heat stability	Induce pink whites of eggs
1	+	+	+	-
2	-	-	++	+

The results indicate that the pink-white factor, which also induced elevated levels of stearic acid and depressed levels of oleic acid need not be associated with a positive Halphen test. It is thus obvious that identification of the biologically active, Halphen negative factor is awaited with much interest. It is of course possible to provide a logical answer for the above results but personal opinion is not always a good substitute for sound scientific data.

Summary

Cyclopropenoid fatty acids, when fed at relatively high levels, appear to induce undesirable effects in animals including: (a) changes in membrane permeability and/or yolk structure which lead to egg discoloration, (b) altered fatty acid ratios, (c) increased chick embryo mortality, and (d) retarded development of reproductive organs. There appears to be little evidence to indicate that ingestion of practical levels of cottonseed products will result in serious metabolic disturbances but pink discoloration of stored eggs will result unless meals of low oil content are selected for inclusion in laying hen rations.

Although an apparent relationship exists between (a) Halphen test, (b) the cyclopropenylene configuration of fatty acids, and (c) unusual biological activity, recent studies indicate that materials other than cyclopropenoid compounds may be responsible for accelerated pink-white discoloration of eggs, associated changes, and altered stearic: oleic acid ratios. Solution of the above enigma should help to clarify the true role of cyclopropenoid fatty acids in utilization of cottonseed products.

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DISCUSSION

V. L. Frampton: I think that the work reported by Evans from Michigan is quite sound. The evidence that we have obtained here in New Orleans is that there is an increased ratio of stearic to oleic and stearic linoleic acid in the cottonseed oils from which the halphen has been eliminated so that the effect on the ratio of these acids is not necessarily to the malvalic acid.

Now, another thing I think is important at this time is the question of the significance and importance of the difference in ratio of stearic to oleic and stearic linoleic acids in the tissues and in the oil of the cottonseed. The question is, is this difference important.

R. A. Phelps: I cannot add very much to that, Vernon, other than the fact that Bob Evans, from data I have seen, has always had the stearic to oleic acid ratio change whenever he has had pink discoloration. As far as linoleic acid content is concerned, this seems to depend upon linoleic acid content of the particular ration fed. This is shown in his published data (Poultry Science 42:875-881, 1963). The same data also show that when he adds corn or olive oil to the cottonseed oil but maintains the same level of cottonseed oil as he had in the previous experimental lot he will maintain approximately the same stearic acid level, but the linoleic acid levels observed in the egg yolks will be governed, at least in part, by the linoleic acid intake in the diet.

Max Milner: What have been some recent applications, if any, to practical oil refining and treatment problems?

R. A. Phelps: There has been a lot of activity but I do not have very much information because most of it is confined to records of the private companies involved. It seems important to point out that the levels of apparent cyclopropenoids in cottonseed salad oil are so low that we do not know anybody that can detect them with accuracy.

E. L. Skau: We now have a method which will permit the determination of the cyclopropenoid fatty acid content of cottonseed oils to within one-hundredth of a percent. We analyzed 12 name-brand cottonseed salad oils and found that they contained from 0.04 to 0.45 percent cyclopropenoids, calculated as malvalic acid. Crude oils run from about 0.5 to 1.0 percent. We have evidence which shows that in cottonseed oil processing the greatest reduction in cyclopropenoid content takes place during the deodorization step.

V. L. Frampton: In partial answer to your question, Max, we have been able to eliminate the halphen response and much of the physiological effects that Dr. Phelps has described through a process of bleaching, using activated alumina that has been treated with sulfurous acid. The second paper in this work is now in press. The third paper is in preparation. We can, in effect and very simply and economically, eliminate much of the effect of these materials in cottonseed oil.

H. W. Bruins: What has been done on the metabolism or the effects of these materials on the metabolism of the fatty acids? Why do you have the increase in stearic acid and the reduction in oleic acid? Is this de novo synthesis of stearic acid or are we blocking some other mechanism which ordinarily produces the oleic acid? Does anybody have any information on this type of thing?

R. A. Phelps: I think we do not really know. Bob Evans is working on this. In Fed. Proc. 21:392, 1962, Dr. Evans and his associates suggest that cottonseed oil interferes with the hen's lipid metabolism so that linoleic acid is converted to stearic acid rather than oleic acid. Others believe that cyclopropenoid fatty acids interfere with the desaturation of stearic acid to oleic acid.

ADVENTITIOUS TOXIC FACTORS IN PROTEINS CONCENTRATES

by
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The dictionary defines "adventitious" as: "Coming from an outside source; additional; foreign; casual; accidental". This seems clear enough. But how shall we consider gossypol when an outside factor such as rainfall influences the gossypol content of cottonseed? Should selenium in wheat be considered an adventitious component? What about arsenic in shellfish? Obviously, as in other situations, things are not always either black or white. However, for our purpose we may agree that although outside factors may influence the quantitative composition of an organism, those components characteristic of normal development and apparently genetically determined are not adventitious. Those components, however, that vary from practically nothing to relatively large amounts, and that depend for their presence on the availability of specific substances in the surrounding soils or waters, are foreign. Thus, we are not concerned in this discussion with gossypol in cottonseed meal, or with the trypsin inhibitor or the hemaglutinin of soybeans, but we are interested, for example, in selenium, arsenic and molybdenum, even though these occur naturally as components of certain species.

The difficulty of defining a toxic factor is exemplified by the spice, nutmeg. In small quantities it is used as a condiment, apparently without hazard, but in large amounts, e.g., a whole nutmeg, it is reported to show marked poisonous properties (1). The toxicity, or capacity of a substance to do harm, is usually expressed as the LD_{50}. The commonly used terms describing toxicity, such as extremely toxic, highly toxic, etc., are associated with rat LD_{50}s varying from 1 mg or less/kg for extremely toxic to 5g/kg for slightly toxic. Anything with an LD_{50} of 15/kg or greater is considered to be relatively harmless. LD_{50} values, however, are not too useful as indicators of chronic toxicity or carcinogenicity. In animal feeds we are concerned both with acute or subacute toxicity that results in unexpected mortality and economic loss. Chronic effects, obviously, are also of crucial interest where long-term or lifetime feeding is involved. In human food, acute effects are easier to identify and to avoid, whereas chronic toxicity and carcinogenicity are much more difficult to evaluate. Obviously a major concern of any consideration of toxic factors in foodstuffs must be with subtle long-term effects.

The term protein concentrate refers not only to materials of high protein content, but to those materials that have had their protein content increased by the removal of a diluting substance such as water, fiber, oil, etc., by some process such as milling, drying, pressing or extraction. The wide range of materials and protein contents included under the term concentrate is illustrated by selected items from the tabulation in Morrison's Feeds and Feeding (2). The highest protein content listed is for blood meal (84.7%) followed by whale meal, herring meal, liver meal, fish meal, tankage and meat scrap, cheese rind or cheese meal, cottonseed flour, etc. At the other end of the scale is low-grade buckwheat feed with a total protein content of 13.3% and a digestible protein of only 5.2%. Wheat bran with 16.9% has slightly more protein than the 15.8% in hard spring wheat that Morrison includes in his tabulation as a concentrate.

Therefore, in discussing "adventitious toxic factors in protein concentrates", we may freely consider any substance in plant or animal raw material that is not a genetically determined component, as well as any substance that gains entry into the final product during its course from the original source to the final consumer.

These components may be derived from natural sources or as the result of man's activities. It is a moot point, whether man has finally caught up with Mother Nature's ability to introduce a diversity of complex substances, capable of causing trouble as adventitious components of human and animal food.

There would be no point in reviewing here the long list of pesticides and food additives that are used in agriculture and food production. The safe use of each of these materials is well understood and controlled by law, so that the residues remaining in accordance with the established tolerances should not represent a hazard to man or domestic animals. However, many of these substances are toxic, and continuous vigilance must be maintained to assure only established safe residue levels. In some cases, a substance of interest as an ingredient in one feed has such great biological activity, that no amount may be tolerated in other feeds. For example, stilbestrol is of some interest as a cattle feed ingredient. On the other hand, extremely low level contamination of other animal feeds can cause serious reproductive difficulties. Hadlow et al. reported (3) mouse feed pelleted in a mill which had been used to pellet cattle feed containing stilbestrol to be responsible for serious reproductive disturbances in the mice. This experience indicates the difficulties of avoiding serious contamination when potent substances are involved, and emphasizes the necessity of adequate precautions and control. Today one would not expect to find deliberate adulteration of food with poisonous materials. Unfortunately, the records of FDA enforcement activities show that this happens much too often. Of particular concern to our discussion is the mixing of fungicide treated seed with untreated seed destined for food use. As you know, the contamination of flour with mercurials from such seed wheat has recently resulted in several deaths overseas. Chronic poisoning in domestic animals from the use of mercurial seed treatments has also been reported (4). It is not always possible to dilute out the poison sufficiently, and in any case such mixing of good and bad products is always an illegal and dangerous practice. In a recent Federal Register publication (5), the FDA announced that after December 31, 1964, it "will regard as adulterated, any interstate shipment of the food seeds, wheat, corn, oats, rye, barley, and sorghum, bearing a poisonous treatment. . . unless such seeds have been adequately denatured by a suitable color to prevent their subsequent inadvertent use as food for man or feed for animals."

There are examples where a compound used in the processing of a foodstuff, although leaving no demonstrable residue, did leave an indelible imprint. In the 1942 Yearbook of Agriculture, in the discussion on the disease of dogs, variously known as running fits, fright disease, or canine hysteria, we read: "no cause is definitely known, though the disease has been attributed to infection, heredity, inbreeding, circulatory disturbances, deficiency of vitamin A or B_1, indigestion, intestinal poisoning, parasites, estrum, and various irritations and excitements" (6). Among suggestions for treatment and prevention are a good diet high in fresh meat, milk, and eggs. In 1946 (7), Mellanby showed that wheat flour treated with the conditioner and bleach, nitrogen trichloride, or agene, produced symptoms in dogs which were indistinguishable from canine hysteria, whereas untreated flour was harmless. The toxic principle, methionine sulphoximine (8, 9), was associated with the protein of the flour and was isolated from treated wheat gluten. This toxic substance, to which dogs and cats are particularly sensitive, interferes among other things with glutamic acid metabolism in brain tissue (10).

Trichloroethylene provides another interesting example of the formation of a toxic derivative by a processing agent. Stockman in 1916 (11) established that soybean meal extracted with trichloroethylene was toxic to cattle, producing an aplastic anemia, whereas extraction with other solvents produced a harmless meal. The solvent, itself, does not produce the toxic syndrome. The active principle is produced by interaction of the trichloroethylene with the protein of the meal, and according to the work of McKinney and his associates (12) is probably S-(dichlorovinyl)-L-cysteine. Trichloroethylene extracted meat scrap is also toxic to cattle.

The fumigant, ethylene oxide, is another example of a substance that produces deleterious effects by reacting chemically with food components. In this case several of the essential nutrients are destroyed, e. g., thiamine, niacin, riboflavin, pyridoxin, folacin, and probably also histidine and methionine. The hazard here is nutritional deficiency disease rather than specific toxicosis (13, 14, 15).

Another example of formation of new substances during processing may be drawn from the field of heated fats. The work of several investigators has shown that fatty acid monomers and dimers, differing from those present in fresh fats and oils in that they do not form urea addition complexes, accumulate during the course of deep fat frying and similar heating conditions (16, 17). These substances have slight acute toxicity. Probably of greater significance is the finding by Kummerow that they seem to be cocarcinogenic in chronic rat feeding studies (18). Since they may be part of the fat portion of protein concentrates their role in any suspected toxicity must be considered. The heated fat situation emphasizes that we know altogether too little about degradation products that may be formed during processing, including the usual home cooking operations. This is particularly true of the various residues of antioxidants, preservatives, emulsifiers, pesticides etc. that are now an inevitable part of our food supply.

Chlorinated naphthalenes provide an example of an unintentional man-made contaminant that produced a new disease first observed in the early forties (19). This new disease of cattle was called "X disease" because of its obscure etiology. In view of the characteristic tissue changes, the term hyperkeratosis is now universally used. This disease has been of great economic importance in the United States; one estimate placed the losses up to 1953 in excess of $20, 000, 000. An extensive research program has pinpointed the chlorinated naphthalenes as the causative agents. The condition has been reproduced experimentally by administering tri-, penta-, hexa-, and octachloronaphthalene. It appears that the higher the degree of chlorination, the greater is the hyperkeratosis-producing property of the compound. These compounds are widely used as components in lubricating oils and in wood preservatives. Many outbreaks of hyperkeratosis have been traced to contamination of feedstuffs, where the lubricating oil in the mixing or pelleting machinery has been allowed to contaminate the feed, or where during harvesting, the oil from the farm machinery has contaminated the crop. It has been shown that concentrates stored in an unventilated room, and exposed to the vapor of a wood preservative containing octachloronaphthalene, can become toxic to cattle. These compounds are highly toxic to all species, and they are excreted in the milk of cows. Undoubtedly after the elucidation of the chlorinated naphthalene-hyperkeratosis relationship there must have been a tendency to ascribe every case of disease to poisoning by these compounds. There is some evidence that even for symptoms of hyperkeratosis there may occasionally be a different etiology. Infestation by toxic species of Aspergillus have been implicated in at least two cases by Forgacs and his associates (20).

Another case of what is probably man-made contamination of foodstuffs and which has not yet been completely clarified is that of the "chick-edema" disease or "hydropericardium factor." In 1957, an epizootic disease caused millions of dollars in losses among broiler flocks throughout a large part of the U. S. After successive elimination of all other possibilities, the etiologic factor was shown to be a toxic fat or a toxic substance in the fat of the ration. The characteristic symptoms were droopiness, ruffled feathers, labored breathing, and high morbidity and mortality. Autopsy findings revealed hydropericardium, abdominal ascites, subcutaneous edema, swollen liver, swollen and pale kidneys, etc. Laying hens showed a rapid drop in egg production. Hydropericardium, the most common lesion in young birds, was not found in birds of laying age. Toxic fat has been fed to guinea pigs, dogs pigs, rats and monkeys with the production of deleterious effects (21, 22). Crystalline substances in a high state of purity have been isolated in three different laboratories and seem to account for all the observed toxicity. These produce symptoms of the disease in young chicks at 50 ppb in the diet and in the original toxic fat were present only in amounts of 1 ppm (23 - 25). The purified toxic factor has also been administered to monkeys. Although the small amount of material available did not permit a thorough study, the preliminary results indicated that this factor is extremely toxic to monkeys. The effects of feeding are irreversible, as evidenced by the fact that the animals succumbed weeks after administration of the substance was discontinued (26). The most recent information from the Procter & Gamble Laboratories on the chemistry of "hydropericardium factor" indicates a hexachlorohexahydrophenanthrene (25). The source of this contaminant is still unknown, but one might infer from its chemistry that it may be a byproduct of production of chlorinated insecticide or other chlorinated compounds. O'Dell found that the chlorinated biphenyls known as arachlors also produced the same syndrome (27). Several arachlors of different chlorine content were tested. Some were not toxic, and others produced the disease at relatively high dosage levels, e. g., 200 ppm. An impurity in these compounds may be the toxic agent or the compounds themselves may be inherently less toxic. Of interest is that the arachlors have been used in some insecticide formulations. It should be pointed out that the toxic factor has occurred in products derived entirely from oilseed sources. Although there is no record of a chick edema positive material except for products of the fat splitting industry, the possibility of the presence or appearance of this factor in physiologically significant amounts in other products, such as protein concentrates, should not be overlooked.

Let us leave man-made factors and consider Mother Nature's activities. For many years, beginning before the Civil War, diseases of animals in certain sections of the Middle West were studied and attributed to various causes. These were of two general types eventually called "alkali" disease and "blind staggers". The early observers were correct in relating the disease to pasturage and to water. In 1927, Franke (28) showed that the disease was related to the consumption of grain grown on certain soils. Cooperative investigations between the South Dakota Agricultural Experiment Station and several bureaus in the USDA revealed the significance of selenium as a toxic factor in food (29, 30). Selenium occurs in the soil both in inorganic and organic compounds, not all of which are available to plants. Where selenium poisoning occurs, the Se content of the soil is about 1-6 ppm, although values as high as 324 ppm have been reported. The danger to livestock results from the accumulation of Se by certain plants. Such accumulator plants, also called indicator plants, thrive best on seleniferous soils and commonly contain 2000-6000 ppm of Se, with values as high as 15, 000 ppm also reported. These seleniferous weeds, in addition to causing selenium poisoning when eaten, also contribute indirectly. They can take up Se which seems to be unavailable to ordinary forage plants, and convert it to a form which, when returned to the soil by their decomposition, can be absorbed by the forage plants. Thus, cereal grains grown on such areas will contain toxic amounts of Se. The organic selenium compounds in such toxic grains are about twice as toxic as the inorganic selenides or selenites. Selenium in animal feeds should not exceed 5 ppm. It is interesting that Se has achieved the status of an essential nutrient, at least for cattle and sheep, and in some areas Se deficiency is an important problem. The required level of about 0.01 ppm is well below any hazardous level. The possible carcinogenicity of Se compounds is still an unresolved paradox. The exact mechanism of the toxic action of selenium is not understood. It may replace sulphur in essential proteins and thus inhibit essential enzyme activity.

Another set of toxic factors derived from the soil are nitrates and nitrites (31). Nitrate has been shown to be the toxic principle responsible for "oat hay poisoning." Many weeds contain dangerous amounts. Unfavorable growing conditions such as periods of drought may result in accumulation of toxic amounts of nitrates in barley, wheat, rye and other common foodstuffs. Attention has been drawn to the possible danger of the use of the herbicide 2, 4-D since sublethal doses of this may have the same effect. Nitrates, although relatively non-toxic per se, may be converted, either in the food, or in the GI tract to nitrites, which produce a dangerous methemoglobinemia.

Another potential problem for protein concentrates arises from contamination of the desired crop under cultivation, with some of the undesired "thorns and thistles" (32). For example, "Wheat contaminated with noxious weeds such as Senecio has caused what has been called 'bread poisoning'" (33). The condition produced in man and animals by plant species containing pyrollizidine alkaloids has been given the generic name "seneciosis". In animals this worldwide disease has been known for years. It has been described in horses, cattle, sheep, goats, pigs, chickens, quail and pigeons (34). In man it has been known as hepatic veno-occlusive disease, and has been a particularly troublesome problem among "bush tea" drinkers in Jamaica (35).

There are numerous species of Crotalaria, a member of the legume family, that have proved poisonous. Crotalaria spectabilis, said to be the most toxic of this species, has been used extensively as a cover crop in light soils in the southern U. S. A. and has spread and become wild in these areas. The toxic principle is the alkaloid, monocrotaline, which produces chronic poisoning for which there is no cure, and death may occur even after intake of the toxin is stopped. Mechanical harvesting equipment recently introduced into the areas where Crotalaria proliferated did not allow the separation of the smaller Crotalaria seeds from those of the cultivated crop. As a result, corn, soybeans, etc., were contaminated and presented a serious problem. In chickens Crotalaria seeds produced hydropericardium similar to that observed with the "chick edema" factor. The potential of such contamination as a source of toxic factors in protein concentrates is illustrated by this item from the Annual Report of the Food and Drug Administration, HEW, for 1962: "This last report discussed the seizure of 47 carloads -- a total of 2, 322 tons -- of soybeans contaminated with poisonous Crotalaria seeds as a result of the growing of Crotalaria plants to improve sandy soils. Only one carload, containing 53 tons, was located and seized in 1962" (36). Apparently the problem is slowly being solved.

It has long been known that many species of the more primitive forms of plant life are of toxicological importance. Poisoning by algae has been reported from South Africa, Canada and the U. S. A. The blue-green algae are almost invariably implicated (37). The species which has received the most attention is Microcystis toxica Stephens, indigenous to South Africa. In acute poisoning, animals die within a few hours. In less severe cases, extensive liver damage, associated with icterus and photosensitization has been seen. The hepatotoxin is an alkaloid.

Ergotism is a chronic illness in some countries due to ingestion of grains, especially rye, which have become infested with a fungus, Claviceps purpurea (38). It has been suggested that some of the plagues during medieval times may have been ergotism. Only a few years ago, several people died of ergot poisoning in Europe. Ergotism is still an important problem in veterinary toxicology. For example, ergot is widely distributed throughout Britain and can be found contaminating a great variety of grasses in varying degrees almost anywhere (39). It is often not clear whether the harmful effects observed from mouldy forage are due to the molds themselves or to the metabolic products they produce. In addition to ergot, where the toxic effects are due to a group of alkaloids and amines, there has been accumulating in recent years a series of examples where the poisonous agents are extracts of the fungal growths. These have been ably reviewed recently by Forgacs (39) and a partial list is worth presenting.

Mold	Food Infected	Toxin	Reported Susceptibility	Symptoms
Sclerotinia sclerotiorum	celery	8-methoxy psoralen	man rabbits mice	blistering lesions of skin exposed to sunlight (365 mμ)
Fusarium sporotrichioides	cereal grain (millet)	unknown	man cat guinea pig dog monkey	"alimentary toxic aleukia"--hemorrhages of skin & mucous membranes, necrotic ulcers in oral & pharyngeal tissues, leukopenia, anemia, fever, bone marrow exhaustion
Sporodesmium bakeri	rye grass bermuda grass	Sporodesium $(C_{19}H_{21}O_6$ $N_3S_2Cl)$ probable	sheep cattle guinea pig rabbit mouse	"facial eczema in ruminants"--hyperirritability, lacrimation, nasal discharge, photosensitization, icterus, stenosis & obliteration of bile ducts, cirrhosis
Stachybotrys atra	hay straw grain	stable to heat, radiation & destroyed by alkali	horses cattle mice guinea pig dogs man	stomatitis, inflammation of buccal tissues, thrombocytopenia, prolonged clotting time, fever, leucocytopenia, massive hemorrhages, fatal in 3-4 weeks, dermal inflammation in man
Bovine hyperkeratisis moldy corn toxicosis of swine				
Aspergillus chevaleri	hay grain	unknown	cattle mice rabbits	acute, fatal in 4-5 days, chronic hyperkeratosis
A. clavatus	pellet feed	unknown	rabbits	dermal toxicity
A. fumigatus	fodder	unknown		

Mold	Food Infected	Toxin	Reported Susceptibility	Symptoms
A. flavus *P. rubrum*	corn	unknown	swine mice	anorexia, cachexia, icterus, fatal 1-5 days, profuse hemorrhages in all tissues mortality 25-50%.
Yellowsis rice toxicity				
Penicillium toxicarium	cereal grains rice	unknown	higher vertebrates	ascending paralysis of CNS origin
P. citrinum	rice	citrinin	mice	acute glomerulonephrosis, liver damage
P. rugulosum		rugulosin	mice rats	fatty degeneration of liver, kidney damage
P. islandicum		chloride containing peptide (H_2O soluble) luteoskyrin (pigment, H_2O insoluble)	rats	fatty degeneration of liver, bile duct hyperplasia, focal necrosis & hemorrhages of liver, primary malignant hepatomas
A. flavus Link ex Fries	peanuts grains	aflatoxins	turkeys ducks swine calves rats	liver parenchymal cell damage, bile damage, bile duct proliferation, hepatoma

The aflatoxin story has focused attention on the important role that field and storage fungi may play, in causing losses among domestic animals, and has underscored the great potential of mycotoxins for contamination of human food. To review briefly (40), an apparently new disease that caused great losses among turkey poults appeared in England in 1960. The causative agent was quickly traced to a feed ingredient, a peanut meal from Brazil. The outbreaks continued into 1961 and shipments of peanut meal from Nigeria, French West Africa, Uganda, Gambia and India were added to the list of toxic feeds. Ducklings, pigs and calves were also affected. The toxic agent, extractable by methanol and soluble in chloroform, was demonstrated to be produced by *Aspergillus flavus* Link ex Fries, a variant of a common storage mold. It is noteworthy that of more than 100 *A. flavus* isolates obtained from a toxic Uganda peanut meal, only 10% produced the fluorescent aflatoxins. Investigators in several laboratories in Europe, and in this country, succeeded in isolating crystalline substances that are extremely toxic, and seem to account completely for the effects of the contaminated peanut meals (41-43). Workers at M. I. T., using a substantial sample of crude aflatoxins provided by the U. S. Food and Drug Administration, succeeded in characterizing and proposing chemical structures for aflatoxins B_1, B_2, G_1, and G_2 (44). The toxic peanut meals in England were reported to be carcinogenic in rats, producing liver carcinomas with metastases in approximately six months (45). The dosage, based on fluorescence assay, was of the order of 5 ppm in the feed. Recent unpublished reports of work in progress indicate that pure aflatoxin B_1 will produce similar results in much smaller dosage. In the United States, Salmon and Newberne (46) have reported the production of liver tumors in rats fed peanut meals produced in the southeastern United States. However, there is still some question as to the identity of aflatoxin with the toxic factor in these studies. A recent communication by Diener et al. (47) reports the isolation of A. flavus Link ex Fries from toxic peanut meals at Auburn University, Alabama.

The most recent food-associated epizootic disease of obscure etiology is rainbow trout hepatoma. Last May (48), the California State Department of Health announced that cottonseed meal was being eliminated as an ingredient of dry fish food, in all California hatcheries. Evidence pointed to cottonseed meal, or something in the meal, as being the causative factor in the high incidence of trout hepatoma in the state fish hatcheries. The data supporting this conclusion have been published recently (49). However, all the available evidence including the information that has been gathered informally does not give too strong support for the implications that cottonseed meal is always the primary suspect, or that other feed ingredients are always blameless. Nevertheless, the basic suitability of cottonseed meal for food or feed use has been questioned. It is therefore necessary to establish the wholesomeness of the basic product, and to develop the specifications necessary to assure the desired quality of the product for food or specific feed use.

The elucidation of the Turkey-X disease epizootic, and the rapid developments in the aflatoxin story, have naturally focused attention on the possibility that the trout hepatoma disease has a similar etiology. Halver et al. (50, 51) have shown that a large number of agents, carcinogenic in the rat, also produce trout hepatoma at comparable dietary levels in fish diets. Preliminary findings indicate that crystalline aflatoxin B_1 will produce trout hepatoma at a dietary level of a very few parts per billion. The carcinogenicity in rats of aflatoxin B_1 is under intensive study. Insufficient data are available to determine whether trout are more susceptible than rats. It is clear, however, that we shall have to increase the sensitivity of aflatoxin assays to a fraction of a part per billion before we can evaluate the significance of aflatoxin contamination as a factor in the trout hepatoma problem.

As the antibotic and fermentation industries have grown since the war, many of the large numbers of microbial metabolites studied have been recognized as highly toxic. The role of fungi as a hazard to food quality, both technologically (52) as well as from the viewpoint of food safety, has been appreciated only recently. Christiansen's studies at Minnesota indicate that some effects of molds, i. e., the lowering or complete destruction of the ability of seeds to germinate, can be observed even in the absence of obvious mold damage, or heavy mold contamination. (53). It would be interesting to determine whether a degree of invasion by a toxin producing strain of A. flavus, just sufficient to markedly affect seed germination, would result in measurable contamination with aflatoxin, or conversely, whether low-level contamination with aflatoxin, would affect seed germination.

The ubiquity of field and storage fungi, our dependence upon harvesting, transportation, and storage of our grains and oilseeds in large bulk, and the increasing knowledge of the destructive and dangerous aspects of mold infestation, pose a serious problem squarely for all of our food industries, and an important challenge to food scientists and technologists.

A discussion of toxic factors would be incomplete without mention of sanitation. The problem of contamination of some of our foodstuffs by Salmonella exemplifies the importance of this factor. That food and feed, particularly when contaminated by filth and insects, can serve as efficient vectors for disease is well known, and is one of the first possibilities to be considered when investigation of a new epizootic or epidemic disease is begun.

After countless generations man has learned that relatively few of the multitudinous herbs, seeds, and fruit may be used for food with safety. Noxious weeds were recognized in Biblical times, when the sowing of tares was regarded as an unfriendly act, as is dramatized for us in the parable from St. Matthew (XIII, 25), "But while men slept, his enemy came and sowed tares among the wheat." Paul Cannon has pointed out that (54) Nature, while generously supplying our daily food, has kept toxicologists busy since ancient times investigating such naturally occurring toxic agents as hemlock, the poisons in certain mushrooms, the atropin in some members of the nightshade family, the digitoxins of foxglove, the ricins of the castor bean, the cyanogetic glucosides in fruit seeds, the gossypol in cottonseed, the goitrogens in cabbage, kale, and rutabagas, the alkaloids of the loco weed, the toxins in certain fish and shellfish, etc. Man has learned to avoid dangerous foodstuffs or through processing to render them useful and safe. However, the relation of dietary intake of minor food components to old and continuing problems, such as cancer, has only begun to be explored.

Remarkable physiological effects can be produced by extremely small amounts of certain substances. The story of the vitamins illustrates the success possible in the difficult task of detecting, concentrating, and isolating in pure form components present in only parts per million or parts per billion. Always some sort of bioassay or a test that correlates with the bioassay is the key. The same general approach has been used in the isolation and identification of minor, nonnutrient components that exert undesirable effects. The late E. M. Nelson told of how he had obtained the first evidence that the toxic principal in toxic wheat was selenium, by feeding the ash of the wheat to rats, and obtaining the same toxic effects as with the original wheat. A comparison of the inorganic elements in the toxic wheat with samples of nontoxic wheat revealed the high selenium content. The aflatoxin story is a variation of the same theme. The implication of cottonseed meal in the trout hepatoma problem has brought to the fore the question of how to proceed to establish the wholesomeness of the basic product, and in the case of trout food to establish the specifications that will assure the quality of the meal for that specific purpose.

The problems of evaluating food are somewhat different from the evaluation of a pure or relatively homogeneous substance, that can be tested at many times the level of its proposed use. In addition to evaluating nutritive value, which is a relatively straightforward matter, it is important to determine whether any toxic components, adventitious or otherwise, are present, and whether, considering the conditions of use, they represent a hazard to health. On the one hand, it would seem logical to make use of our experience with the isolation and identification of many physiologically active substances in the solution of this latter problem. Instead of depending entirely on responses observed during feeding of the product, as it will be finally consumed, why not administer a series of extracts and concentrates? In this way we would have the advantage of increased dosage in studying the biological response, if any, just as we do in the case of chemical additives. This approach, although possibly more costly, should yield greater dividends in knowledge and assurance of safety. On the other hand, logic would seem to dictate that our efforts should be focused on the specific problems of which we are aware, that our first job would be to isolate the factor from a meal known to produce trout hepatoma, to identify it; to determine its origin, then to decide how its occurrence may be prevented or how it may be neutralized or processed out of the product.

This is a straightforward approach and one difficult to argue against, but it leaves one with the uncomfortable feeling that instead of deliberately setting out to determine the safety problems that may characterize a new food product, or one whose wholesomeness has been questioned, we are depending primarily on experience to do this for us. Of course, when the problem arises and is well delineated, it is much simpler to deal with it. It is much more difficult to hunt for something which may not be there. We have all experienced dissatisfaction and displeasure with negative results. Nevertheless we should not continue to depend on experience alone to uncover problems for us. What is needed, and what we should attempt to develop, is a methodical means of anticipating, detecting, and avoiding potential problems of food safety before they break upon us.

A few examples of adventitious food components and the problems that can result from their presence have been reviewed. Such problems have been occurring since Biblical times. However, as knowledge emerges and scientific skills develop, we are learning to solve them relatively quickly. Although there is still a long way to go before food safety can be evaluated with the desired speed and accuracy, we can anticipate problems by systematic investigation.

Today we are aware of a potential threat of mycotoxins to the food supply. Work is in progress toward the elucidation of the trout hepatoma problem. Developments in these areas have raised serious and difficult questions about the suitability of peanut and cottonseed meals for food and thus challenge two of the most promising protein sources so urgently needed to combat kwashiorkor and protein malnutrition. There is no doubt that food scientists and technologists will meet this challenge, and that just as the inherent toxic factors are being successfully neutralized, the obstacles of the adventitious toxic factors will also be overcome.

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USE STATUS OF COTTONSEED AND OTHER PLANT PROTEINS

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Discussions which have preceded, and others which will follow, give ample assurance that fine basic work is being performed in plant protein research. It is appropriate that we also take a look at the application of these research findings, and discuss the uses that are being made and are likely to be made of cottonseed and other plant proteins.

Some of the trends apparent in the use pattern of plant proteins today shed light on what may be expected in the future. Total production of vegetable protein concentrates in the United States in the year October 1962 to September 1963 amounted to 14, 363, 000 tons (33). (Table I). These include soybean, cottonseed, linseed, peanut, and copra. Of that total, 11, 127, 000 tons were soybeans and 2, 692, 000 tons were cottonseed.

In the usage or disappearance of these concentrates, 12, 620, 000 tons went into animal and poultry feeds. Only 60, 000 tons, divided equally between soybean and cottonseed, went into the category designated "other uses." Animal agriculture, principally through the feed industry, continues to be virtually the sole user of plant protein concentrates in this country. Putting it another way, all formula feeds manufactured by the feed industry in 1962 were estimated at 44 million tons. Approximately 20. 8 percent of this total consisted of soybean meal, and 2. 6 percent was cottonseed meal. This indicates, then, that some 9. 2 million tons of soybean meal and some 1. 1 million tons of cottonseed meal were used directly by the feed industry in the manufacture of formula feeds, or a total of 10. 3 million tons of these two sources alone.

TABLE I

OILSEED CAKES AND MEALS - SUPPLY AND DISTRIBUTION

October 1962 to September 1963

(Thousands Tons)

	PRODUCTION		USES		STOCKS*
		FEEDS	OTHER USES	EXPORTS	
SOYBEAN	11, 127	9, 562	30	1, 476	153
COTTONSEED	2, 692	2, 560	30	85	159
LINSEED	371	320		52	7
PEANUT	68	67			2
COPRA	105	111			
	14, 363	12, 620	60	1, 613	321

*Inventory changes not accounted for.

Against the background of these current usage patterns let us place another trend which is most significant. During the last 15 years, both soybean meal and cottonseed meal have steadily accounted for an increasing share of the total value of the original bean or seed (34). Figure 1 pertains to soybeans. We shall examine similar cottonseed figures in a moment. In 1947, oil accounted for 54. 6 percent of the total soybean value. With minor temporary deviations, this share has steadily dropped and in the 1962-63 year oil represented only 34. 8 percent of the total soybean value. Conversely, the value of meal amounted to 45. 4 percent of the total in 1947. This has risen to 63. 7 percent now.

The same trend, in Figure 2, holds generally true in cottonseed. In 1947, oil accounted for 60. 3 percent of the total cottonseed value (34). This has dropped to 45. 9 percent currently. In 1947, cottonseed meal accounted for 28 percent of the total which has risen to 40. 5 percent now. These percentages do not take into account the linters and hulls.

These current usage figures show us that cottonseed and soybean meal are shouldering a steadily increasing share of the total value of cottonseed and soybeans. They also show what we already know, that practically all of the meal is going into animal feed usage in this country.

The message in these trends seems obvious. Animal and poultry feeds cannot continue to absorb, at favorable price levels, more and more plant protein concentrates. Nor can they continue to assume an ever increasing portion of the total value of soybeans and cottonseed. It is true that the vegetable proteins have been a very important factor in the continued expansion of the feed industry, and consequently, also in making possible much more economical production of meat, milk, and eggs. The brightest future prospects in the plant protein industry lie in the upgrading of the protein so that it may be diverted in increasing volume to human applications, and also to other industrial uses.

Expanding the use of plant protein for human applications is no small order. It involves both basic and applied research of the most imaginative kind. Some fine research in this area has been and is being done. Since this field of application seems to be so important to the future of plant protein, let us consider the current state of its development.

Cottonseed

The use of cottonseed protein for human consumption has been studied and reported almost entirely in terms of low cost diets for use in protein deficient areas of the world, although certain domestic uses of cottonseed flour, such as in donuts, should not be ignored. Its use in Incaparina type formulas is typical. Since several other speakers on the program are to cover these specific areas of research, we can pass over a review of this subject by merely stating that on a worldwide basis this is an important and active area of research which has been recently reviewed by Scrimshaw (28) and by others.

Soybean

Soybean protein which is preeminent as a vegetable protein for animal feeding is attracting increasing interest in the field of human nutrition. Part of this is the result of availability in a variety of forms suitable for use in human foods. Aside from the whole bean used as a vegetable variety, which has had only minor acceptance outside of the Orient, the forms of soybean protein now commercially available are as follows:

1. Soy flours with or without various portions of the original fat, are obtained basically by grinding soybeans or defatted soy flakes. Often a treatment may be introduced such as that of Paulsen (22) who employed a protonic acid plus hydrogen peroxide to reduce flavor and color. One of the problems of the past has been the natural bitter flavor of the soybean, which comes progressively more objectionable, and therefore must be removed or neutralized. As has been mentioned by Bitting,

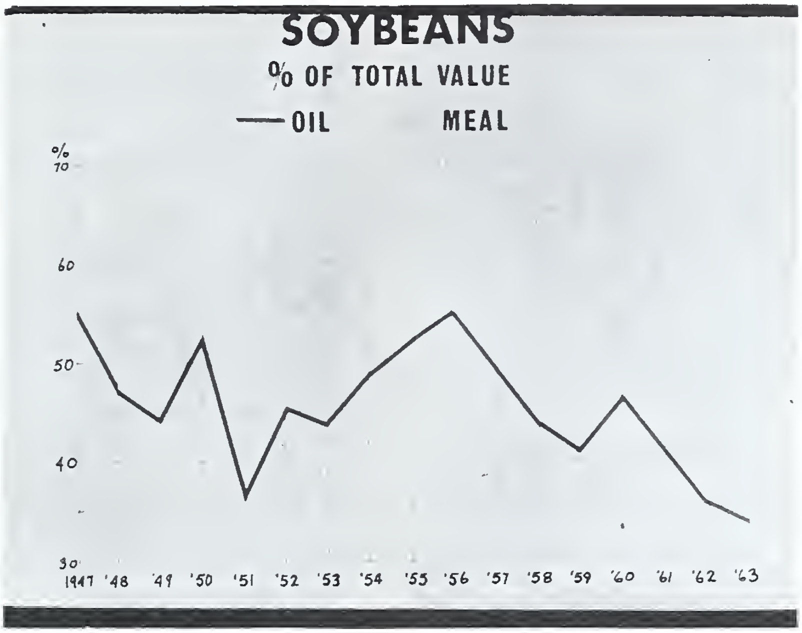

Figure 1.

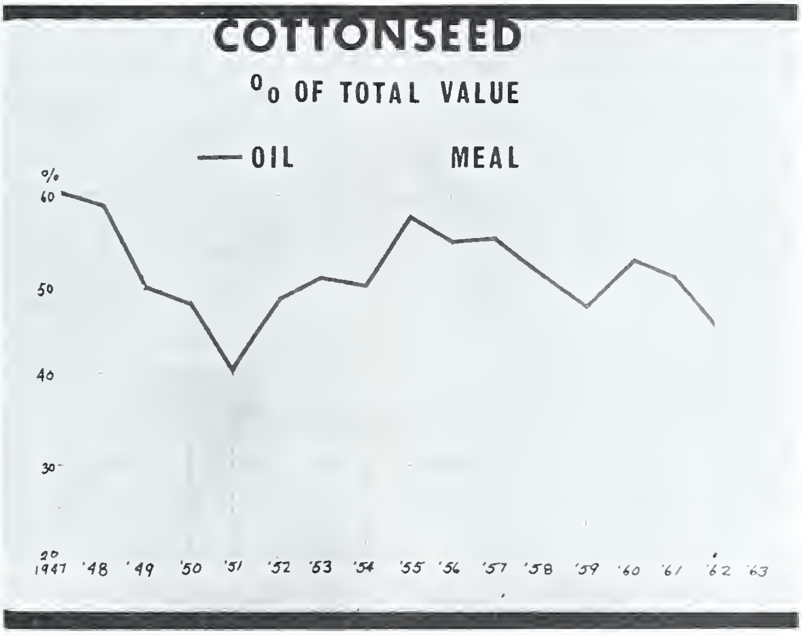

Figure 2.

this is one of the low-cost sources of protein and probably the one best adapted to use in developing populations.

2. Isolated soy proteins are obtained generally by some variation of the basic process first proposed by the Northern Utilization Research and Development Division (Belter, et. al. (4)), a method in which solvent defatted flakes are extracted with an aqueous alkaline medium, extract clarified after separation from the residual flakes, and the soy protein curd then precipitated at its isoelectric point of about 4.5 pH. The curd can be washed to remove carbohydrates and other soluble nonprecipitable components and then dried by any one of several means. For edible use, spray drying is often employed. If desired, the protein curd may be neutralized before drying by any of several alkaline neutralizing agents to form Na, K, Ca, etc. proteinates. Many variations of the above process are possible and numerous patents exist in the field. The exact details of the various commercial processes are not available. Protein content of an isolate runs in excess of 90 percent, (N_2 x 6.25), and more often in the 95 percent plus range.

3. A somewhat less purified form of soybean protein, known as soybean concentrate (protein content approximately 70 percent), has been prepared by several processes which remove the water soluble carbohydrates and the flavor and color components from the extracted flakes, leaving all other seed fractions behind in a residual product. Among the methods to achieve this is the aqueous extraction procedure of Sair (27) at an isoelectric pH or extraction with a polar solvent, such as one of the lower alcohols, at a suitable concentration, as proposed by Mustakas et. al. (19).

4. A bland tasting water soluble form of protein known as Gelsoy (Beckel (3)) is prepared by alcohol washing of hexane extracted flakes, then by aqueous extraction of the dried alcohol washed flakes, followed by recovery of the aqueous extract by spray drying. It has a protein content of about 55 percent and it has special emulsifying properties. Whippable soy proteins have also been prepared by enzyme hydrolysis (Burnett & Gunther (6)) and more recently by alcohol washing of unhydrolyzed acid-precipitated soy protein (Eldridge, et. al., 1963 (11)).

5. A more recent achievement in the soybean protein field is the development of an interesting fibrous texture by a textile-like spinning procedure first proposed by Boyer (5), and later modified in a series of patents. Basically this process consists of taking the soy protein isolate, either as the curd or the isolated powder, dispersing it in alkali, forcing the protein dispersion through small orifices into an acid-salt coagulating bath from which it is withdrawn under tension as a series of fine threads of protein fibers. These, when washed free of the acid-salt brine, are white, bland threads of soybean protein as illustrated in Figure 3.

With soy flours, soy isolates, soy concentrates, soy whipping agents and soy fibers all commercially available it is natural that a great amount of research should be devoted to their utilization. Much of this research is in the form of commercial development. In general, soy protein is used either for the nutritional purpose of raising protein levels in a food or for some useful physical attribute which the soy ingredient may possess. Some of the categories of food uses which have been reported in the literature are baked goods, whips, sausage-like products, soy milks, oriental type foods and simulated vegetarian meats. An excellent review of many of these uses was presented by Smith and Wolf (31) and Wolf and Smith (36) at the 20th annual meeting of the Institute of Food Technologists. Nutritional values for various soybean fractions were discussed in the above reviews.

The use of vegetable proteins for infant feeding has been recently reviewed (Anon.) (1) and the point was made that little well-controlled and reported experimental work has been done in this field despite the use of soy milks for babies allergic to cow's milk in this country, and a general lack of good protein of any type in other areas of the world. Tepley and Gyorgy (32) have discussed the general subject of plant proteins of all types for infant feeding, and more recently Omans, et. al. (21) have reported studies in which four commercial soy milks were compared with cow's milk when fed to young male rats. The soy milks gave protein efficiency ratios only 1/2 - 2/3rds the value of cow's milk. A similar report has been made by the Committee on Nutrition of the American Academy of Pediatrics (10).

Soy flours can be used in a variety of baked goods to raise the protein level. For example, Rusoff et. al. (26) added toasted defatted soy flour to donuts at a level to supply 20 percent protein in the product. Rat tests gave excellent growth results with protein efficiency ratios superior to casein controls. Peanut and cottonseed protein supplementation of donuts gave substantially lower gains and PER (protein efficiency ratio) values. Fish protein gave values close to the casein control but below that for the soy flour.

Raw soy flours at levels of 1 percent are said to improve white bread (Pollock and Geddes (24, 25) (1960)); but higher levels decrease loaf volume. Heated soy flours were more injurious in proportion to the amount of heating. Fractionation of the soy flour by solution, precipitation and dialysis techniques gave a product which, at a 3 percent level, gave poor results but it was improved by heat and higher bromate levels. Learmonth and Wood (17) in discussing the starch liquifying enzyme of raw soy concluded that it was not significant in starch breakdown during fermentation or baking. The anti-proteolytic activity of raw soy is useful in controlling undesirable proteolysis sometimes produced by malt. Heated soy flour can be used up to 5 percent with proper oxidizing agents to prevent loss of loaf volume, according to the work of Ofelt, et. al. (20). Complete soybean foods have been used in the Orient for many years as important parts of the diet. Smith (30) has reviewed their use in Japan and describes the many forms of use in a publication entitled "Use of United States Soybeans in Japan". Recent studies on several products have been reported. Chiu and Van Duyne (9) have described the preparation of tofu, or soybean curd, a cottage cheese like food which they prepared from a number of varieties of soybeans. All varieties studied attained good palatability scores.

More spectacular demonstrations of the use of soy protein in the human diet in forms more easily adapted to the American eating habit are the recent applications of soy protein fibers to the production of simulated high protein foods with natural texture. Examples are shown in Figures 4, 5, 6, 7. This recent development described by Kyd (14) has added natural meatlike fibrous texture to the factors man may control in engineering foods for the future. Kyd describes meatlike products simulating dehydrated chicken cubes, beef, and processed ham, and also a chip type. He points out that not only can natural texture be achieved but that the type and amount of fat and other nutrients can be controlled at the will of the producer. This would appear to give the food technologist all the control he would desire in the matter of nutrition, texture, flavor, and appearance. The possibility of totally new food forms based on plant proteins is held as an attractive goal. A large amount of research by a great many companies is going on in this area and it will be interesting to follow developments.

Sjostrom (29) in introducing a symposium on Foods of the Future stated that the possibilities include "derived foods made from building blocks of isolated or synthetic components; new processing methods that will result in foods that retain their natural characteristics over longer periods; flavoring compounds that will make existing products more acceptable; and foods that will perhaps influence our very physiological and psychological reactions".

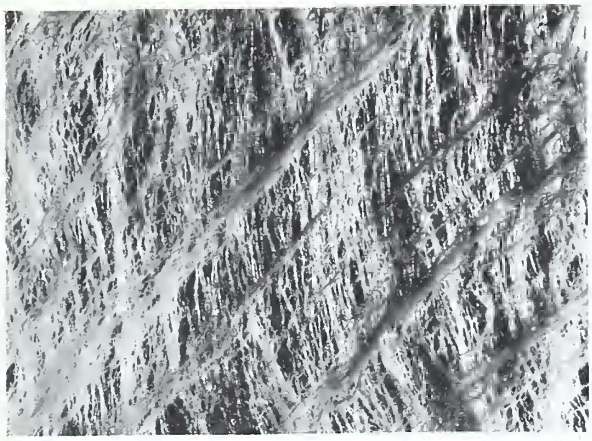

Figure 3.

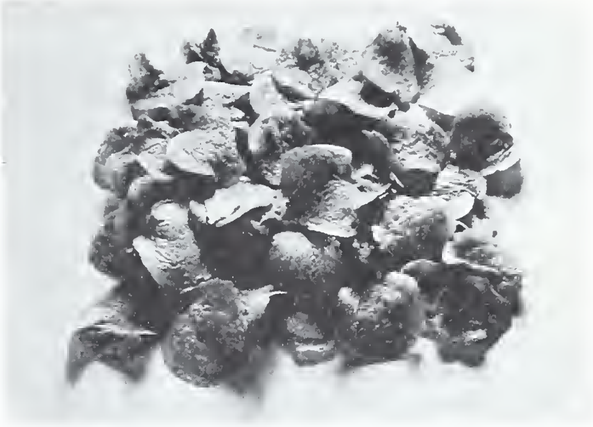

Figure 6.

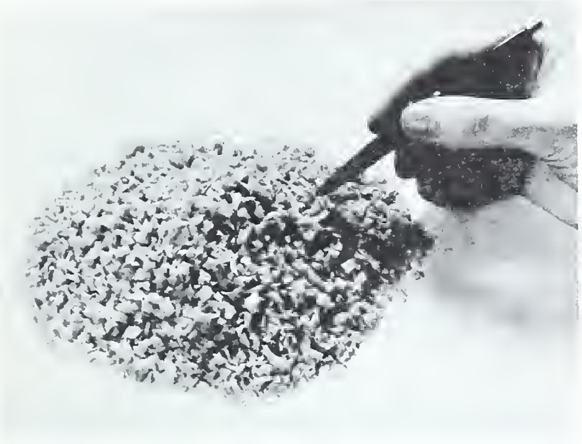

Figure 4.

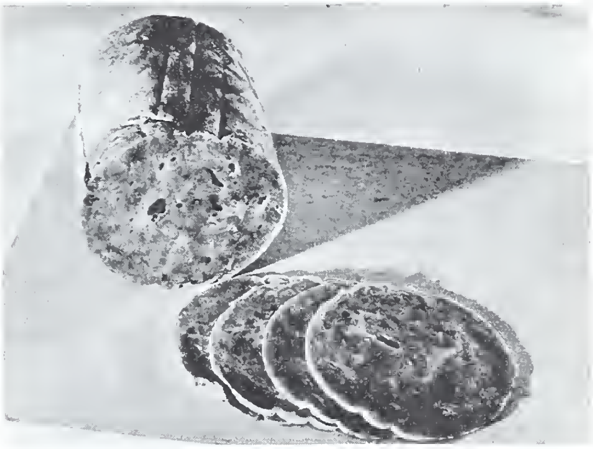

Figure 7.

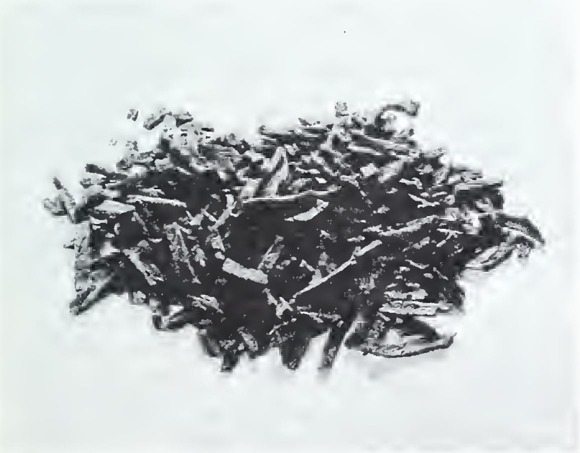

Figure 5.

Vegetable protein isolates and fibers appear admirably to fulfill these conditions since they have useful nutritional and structural properties, are themselves quite stable, and are so bland and light in color that they permit the use of a full range of flavoring and coloring control.

Peanuts

While peanut protein has received less interest for human nutrition in this country than some other sources it has been of greater interest in protein deficient areas where it is available. Milner (18) has reviewed the use of peanuts as a protein resource in international feeding programs such as UNICEF, FAO, and WHO at a conference here in New Orleans some time ago. Joseph et. al. (12, 13) have published recently on the supplementary use of peanuts in blends with skim-milk powder, with and without other supplementary proteins, in corn-tapioca type diets and as a protein addition to diets fed undernourished children in India.

The completion of a commercial plant at Plymouth, England, for the production of peanut lipoprotein or of peanut isolate by the impulse rendering process of Chayen (8) has been the occasion for a series of papers describing the peanut lipoprotein product produced. Waldt (35) describes experiments in which peanut lipoprotein was used to replace a portion of the eggs in yellow cake mixes and in other baked goods. Grading scores were comparable except with regard to color, in which case additional color would be required to replace the natural egg color. Volumes were down about 2-3 percent. In comminuted meat formulations of the frankfurter type, less than 1 percent addition of peanut lipoprotein gave better grading scores than the all meat controls or those with caseinate or milk solids as added binders. The addition of 1.5 percent lipoprotein to an aerosol whipping topping improved texture and stability.

Other Protein Sources

Little work has been reported recently on other plant protein sources with the exception of a considerable interest in algae, particularly for space travel. Casey and Lubitz (7) have summarized the status of algae as a food for space travel in a recent extensive review which should be consulted by those interested in this subject. Lubitz (16) in a study of one strain of Chlorella #71105, found that the freeze dried product contained 55.5 percent protein with a digestibility of 86 percent; PER values were found to be 2.19 as a single protein source with casein at 3.3. Methionine supplementation increased the PER value to 2.9.

Leaf proteins have been suggested for low cost nutrition in protein deficient areas but are receiving little attention, although Pirie (23) found the heat coagulated protein obtained from an aqueous extract to be as good as or better than fish for pigs, rats, and chicks. The Chayen process, now applied to peanuts, was originally proposed for leaf protein extraction, but no recent progress reports have appeared.

Of general interest to researchers studying protein in human nutrition are the recent report of Bandemer and Evans (2) on the amino acid composition of some seeds, and the comprehensive review of Liener (15) on the toxic factors in edible legumes and their elimination. The latter paper includes some 265 references.

The fact that we have confined most of our discussion to research in the human application field does not mean that research is not going forward in the animal field, too. There is fine research here, but the human field offers more dramatic promise at this time.

The plant protein industry is today a vital factor in the production of human food through animal agriculture. In the years ahead it will unquestionably take on a broader and more significant role in total food production in the entire food industry.

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DISCUSSION

Leonard Smith: I would like to make a comment about the protein fibers. And I make this comment since there are those who might get the idea that because gossypol causes problems in the food and feed utilization of cottonseed proteins, the fiber would be a very logical use for such protein. There have been some pretty good fortunes poured down the drain on protein fibers. They have tried to make them of peanuts and milk, among other materials. Probably the most successful was Zein fiber from corn. All of these have failed and they failed at a time when synthetic fibers were selling roughly at a price about twice what they are now.

H. L. Wilcke: I think, Leonard, you are referring to the developments for textiles uses.

Leonard Smith: Yes.

H. L. Wilcke: These uses that we are speaking about here are not for textile use. The fibers are not strong enough for that particular purpose. They would have to be used in food products and not in textile use.

M. L. Anson: Three comments. First, about the increasing use of oilseed proteins for human foods mentioned by Dr. Wilcke. It is commonly said that the great application of oilseed proteins to food will be in the undeveloped countries which need protein but cannot afford animal protein foods. My own opinion is that the price advantage of oilseed protein foods over animal protein foods will also ensure great applications in developed countries.

Second, I think it is about time that the expression of protein content as nitrogen times 6.25 be abandoned, especially before an audience such as this one. The factor of 6.25 is a convention of the feed industry. Such an arbitrary universal factor was abandoned by academic protein chemists as soon as it became clear that the factors are quite different for different proteins. It is sound to express protein as nitrogen times a factor only when the factor is known.

Third, it ought to be emphasized that many of the basic procedures we all use, and which have been discussed, are much older than is often recognized. Although the food-technological applications may be new, the methods of preparing isolated oilseed proteins are quite old. The technique for making protein fibers was understood long before the recent use of such fibers in foods, etc.

N. S. Scrimshaw: Thank you, Dr. Anson. Don't leave the microphone. It may be unnecessary for this audience, but I think it would be worthwhile to put on record just why the nitrogen times 6.25 is not a convenient expression for this particular purpose.

M. L. Anson: The factor 6.25 may be convenient, but it just is not correct. In my own limited experience 5.7 or 5.8 would be more nearly correct for isolated soy protein, anyhow, a factor quite different from 6.25. It is silly, as a result of using a wrong factor, to talk about 70 percent protein that is not 70 percent protein or to report that a preparation of isolated protein is 110 percent protein.

N. S. Scrimshaw: Well, added to this problem is the fact that part of the nitrogen isn't in protein form at all. When one speaks of nitrogen or of conventional protein without specifying its quality, this lessens the interpretability for human or animal nutrition of any nonprotein values which are given.

M. L. Anson: It is certainly true that in some materials such as yeast, a good deal of the nitrogen is not amino acid nitrogen. If one knows only the nitrogen, one should just give nitrogen values. If one knows how much of the nitrogen is from protein, one can talk about protein nitrogen. If one knows the factor, one can then calculate proteins. To get protein nutritional value one needs still other information.

A. K. Smith: May I say a word about this factor? The factor is always based on the nitrogen content of the protein, and in our laboratory when we make acid precipitated protein by the normal procedure from hexane extracted flakes, we get a factor that will be very near to six. Now if you refine and purify the protein the factor will change because of the removal of the impurities present in the protein. If you refine the protein as high as it can be refined your factor will be about 5.75. And if you use alcohol extracted flakes you will have still a different factor. So, you really have to work out a different factor for every type of protein you prepare if you want to use a factor for calculating protein. That is our experience.

H. L. Wilcke: I am glad this discussion came up. In fact, I told someone yesterday I would be very much surprised if it didn't. This has been the subject of much discussion. When definitions were set for these products it was very much a point in question. The fact remains that in defining these products a protein content must be given, and the thing that Allan Smith just mentioned is a very confusing point. How are you going to set a different factor for each one of these products produced with different methods when obviously we are going to get some different factors?

This other point that Tim mentioned is very true. We frequently come up with products that have either over 100 percent protein or would be over 100 percent when you figure the ash and the moisture. It is something that needs correction. So far, there has been no good solution presented.

A. K. Smith: May I say one more word, please? We have been asked many times to work out the correct factor for calculating protein for soybean meal. I doubt that such a factor would be very useful. U. S. industry has always used the factor of 6.25. They are accustomed to it. Perhaps there is an error involved in the use of 6.25 but the whole industry has adjusted to that error, so there is not much point in spending a lot of time and a lot of money trying to work out a factor which they probably would never use.

A. M. Altschul: May I make a comment? The point is that seed proteins in general have very high glutamine content, and so they have large amounts of amide nitrogen. And the amount of amide nitrogen might vary depending upon the condition of processing and handling, because this is sort of labile nitrogen. And so you can see that if you multiply nitrogen by some factor, even if the factor was obtained once on a protein, you might not get it again on a particular protein. So that supports the point of view of Anson.

The biochemists who report protein content of preparations have been having considerable success with the Lowry technique, which is a modified Bieuret reaction. This what we use when we talk about protein content of a preparation. I don't know how the Lowry technique would work on mixtures. Again it depends upon the kind of protein. It might be a very useful way of measuring protein content.

Max Milner: I missed in Dr. Wilcke's talk where he was referring to nutritive values of various types of derivative soy products, the effect of supplementation with methionine in relation to nutritive value. I believe there has been some published work on this. I realize that there are some unsettled questions as to the requirements for methionine in various species, that most of these tests I refer to have been done with animals, and that some may have higher methionine requirements than others. I wonder if Dr. Wilcke would care to comment on the supplementary effects of methionine on the nutritive quality of derivative soy products.

H. L. Wilcke: I did mention that on one of them, Max. It is obvious, I think, and it has been demonstrated many times, that methionine supplementation will improve these products and that most of them are deficient when used as a sole source of protein.

Now, this brings up another interesting point that when one of these isolates was used as a supplementary protein it gave a very high PER. This, I think, is also the experience when you use it with other proteins. It can be combined to correct these deficiencies.

N. S. Scrimshaw: We will return to some of this in the later discussion. I think it is noteworthy that 15 years ago there would have been very much less interest in the kind of discussion we are having this morning. In fact, it was only in the early 50's that food and nutrition workers in the developed countries began to realize that there was a world shortage of protein for human consumption and that this problem was not going to be solved entirely by conventional means. If they had considered it before, it was in terms of increasing the production of milk and meat and eggs wherever protein was needed, with some exceptions, of course. But in the early 50's WHO, FAO and UNICEF became increasingly aware of the problem, and increasingly stimulated world recognition of it.

Contributing to this understanding was the work of the Institute of Nutrition of Central America and Panama (INCAP). In Central America, as in other technically undeveloped areas of the world, the people in the lower socio-economic groups were not getting adequate amounts of protein. The children were grossly smaller and had a much higher morbidity and mortality than their counterparts in more highly developed countries. It soon became apparent that even if the availability of milk, meat, and eggs could be doubled or tripled, the quantities of protein thus provided would still fall short of the need, and furthermore, the purchasing power of the people was so low that they would not be able to take advantage of such increases in quantity. A lower cost had to be found, and a more practical solution than just increasing the availability of animal products.

It is interesting that it was the work of a poultry nutritionist, Dr. Robert Squibb, looking for indigenous sources of protein feeds for animals in Central America, combined with the interest of INCAP in human nutrition that led to the initial development of a formula called INCAP Vegetable Mixture 8 which used 9 percent cottonseed flour and 28 percent sesame flour. This formula went through all the stages of laboratory testing, and even preliminary field testing before it became apparent that although it was a nutritional success, it would be an economic failure. It was impractical because of increase in the cost of sesame and of certain problems associated with the processing of sesame.

It was at this time that Dr. Ricardo Bressani, who had previously been with INCAP after receiving his Masters Degree in Agricultural Chemistry at Iowa State, returned after receiving his Ph.D. in Agricultural and Food Chemistry at Purdue University. He plunged with enthusiasm into the problem of finding a practical formula. By that time we knew a bit more about cottonseed and cottonseed flour and had more confidence in it. To him should go the credit for the present basic formula of Incaparina, Mixture 9, and the vigorous laboratory and animal testing program which followed. At this meeting he will be telling you of additional formulas along the lines of Formulas 8 and 9.

It was recognized that mixtures of this sort had to have a name, and with considerable hesitation, the initials of the Institute, INCAP were coupled with the Spanish word "Harina". Incaparina is a generic name for vegetable mixtures containing over 25 percent protein of relatively high biological value developed by INCAP and suitable for human consumption. I think that this is sufficient introduction for Dr. Bressani, who since 1956 has served as Chief of the Division of Agricultural and Food Chemistry of INCAP and who, this year, is serving as Associate Visiting Professor in the Department of Nutrition and Food Science at M.I.T. He will be returning to his post in Guatemala at the end of this academic year.

TECNOLOGICAL ASPECTS OF INCAP VEGETABLE MIXTURE NINE

(Incaparina)

by
R. Bressani, E. Braham, L. G. Elias and R. Jarquin
(Presented by R. Bressani)

Introduction

Cottonseed flour has become a potential source of good quality protein for human consumption particularly in those areas of the world where the intake of protein, both from animal and vegetable origin, is not sufficient to meet the daily requirements of the population. INCAP Vegetable Mixture 9 (1, 13), known as Incaparina, has been successfully introduced as a protein supplement to several populations in Latin America whose animal protein intake is low. The percentage composition is cottonseed flour 38, corn flour 58, torula yeast 3, calcium carbonate 1, and 4500 I.U. of vitamin A per 100 g (1, 13). Chemical and amino acid analysis have indicated that the mixture contains 27.5% protein, 4% fat, and an adequate amino acid pattern (1, 13). The formula has been extensively tested in chicks (2), rats (3), dogs and swine (4), and children (5, 6, 14). The results of these studies indicate that the average PER (protein efficiency ratio) is 2.20 in rats (3) and the biological value is 72.5% in dogs (4). At low levels of intake the nutritive value is slightly lower than animal protein such as casein, milk, and meat (3). Amino acid supplementation studies in rats suggested, however, that at low levels of dietary protein, Mixture 9 becomes limiting in lysine and threonine (7). The average nitrogen retention in children fed 2.0-2.9 protein/Kg of body weight per day from Vegetable Mixture 9 was 17.8% of the nitrogen intake as compared to 16.3% for skim milk fed at the same protein level with protein digestibilities of 68.9 and 82.6%, respectively (5, 6, 13). Biological tests were also carried out to study the effect of several levels of protein in the diet from Vegetable Mixture 9, casein and skim milk on the carcass, liver, blood and bone composition of growing rats (8). At low or high levels of protein intake, body and liver fat concentrations were higher in the animals fed the Vegetable Mixture. At low levels of protein intake higher values for total serum protein, albumin, urea nitrogen concentration as well as femur and tibia fresh weight and bone ash were observed in rats fed skim milk. The values became similar when protein intake was increased, or when the amino acid deficiencies in Mixture 9 occurring at low protein intake levels were corrected by supplementation. Studies in rats of the supplementary effect of Mixture 9 on three different Guatemalan rural diets which are fed to children have shown that a significant improvement in weight gain and in protein quality was obtained by feeding 4.0 g per day of Mixture 9 (9) as a dietary supplement.

From extensive experimental testing, it became evident that the mixture is of good quality and is free of adverse physiological effects. Furthermore, it is capable of supplementing adequately diets of poor quality and is acceptable by the population for whom it was intended. Since commercial introduction in 1959, several problems have arisen, however, particularly with respect to the production and availability of a cottonseed flour of a high protein quality and an acceptable free gossypol content. One source was located in El Salvador which was usually good but occasionally failed to meet specifications. The results to be described here summarize part of the work carried out since 1960 on several technological aspects of Vegetable Mixture 9, especially those affecting the nutritive quality of cottonseed flour.

Studies on Cottonseed

There are several reported studies on the factors affecting the gossypol content of cottonseed (10). These, however, are from regions other than Central America. It was of interest, therefore, to determine the variability in chemical composition and gossypol content of varieties grown in this area, as well as to learn the effect of locality and other environmental factors on the pertinent chemical components of the cottonseed kernel. These studies partially summarized are shown in Table I(11). Thirty varieties grown in one locality and 264 samples of one variety grown in four localities were analyzed for their proximate chemical composition, gossypol and lysine. Only the ether extract, nitrogen, lysine and gossypol values are presented. The samples were collected directly in the field in cotton plantations from four agricultural areas in Guatemala. The results show that gossypol varied from 0.697 to 1.162% in 30 varieties, while the variation between the four localities for one variety was from 0.660 to 1.288%. In general, samples coming from the areas of less moisture and higher temperature contained less gossypol. With the development of gossypol-free cottonseed it might appear that there is no need to study the variability of the pigment in cottonseed. However, the widespread introduction of such seed is in the uncertain future.

Studies on the Production of Cottonseed Oil Meals

One of the most important problems facing the production of INCAP Vegetable Mixture 9 is the availability of a high quality cottonseed flour. So far the commercial production has been made with cottonseed flour from a cottonseed oil mill using a prepress solvent extraction process (13). However, the flour has not always met the specifications set by the 1960 Cottonseed Conference (12) with respect to the amount of free gossypol, and many lots have been rejected. Although suggestions had been made concerning the process to be followed for the production of a suitable flour, initial attempts to apply these suggestions have failed. The failure was not necessarily due to any lack of validity of the suggestions, which were good and logical, but it was due to the fact that the mills were designed primarily for the production of oil rather than flours. Furthermore, there were many instances in which the mill engineers showed a lack of understanding of the necessity to produce a good quality flour. In view of the above, studies were carried out to ascertain the important factors affecting

TABLE I. Effect of Variety and Locality on the Ether Extract, Nitrogen, Lysine and Gossypol Content of Cottonseed

Sample	Number of Samples	Ether Extract %	Nitrogen %	Lysine %	Gossypol %
Varieties					
Range		21.8-28.5	3.53-4.30	0.550-0.833	0.697-1.162
Ave.	30	25.2	3.82	0.720	0.931
Localities					
I Range		19.9-26.5	2.99-4.24	0.647-1.020	0.704-1.116
Ave.	85	23.9	3.65	0.789	0.882
II Range		20.1-25.8	3.35-3.88	0.682-0.920	0.712-1.042
Ave.	33	23.4	3.65	0.793	0.857
III Range		19.2-26.6	3.36-4.07	0.732-0.982	0.660-1.288
Ave.	90	23.9	3.38	0.822	0.919
IV Range		17.7-26.8	3.01-4.16	0.693-0.909	0.646-1.063
Ave.	56	22.9	3.56	0.806	0.848

gossypol and available lysine during the processing of cottonseed. Results from these studies are summarized in Table II (11). Four cottonseed oil meals in Central America using the expeller process were visited at least twice and one using a prepress solvent extraction process was visited at least 6 times. Representative samples from seed to flour, including samples from all other important operations were obtained together with information on the existing operating conditions of temperature and time. In the Table the results show that only the material produced from the prepress solvent extraction mill gave a flour of good quality even though it tended to have a high free gossypol content. Important findings were a) that the moisture content during cooking of the seed which was about 10% is not high enough, b) that in many cases most of the gossypol was already bound before cooking and extraction, c) that temperatures, particularly in the conditioner were relatively high, and d) that the seed used was not necessarily of the best quality. Studies confirming observations of other workers (10) indicated that cooking at higher moisture levels decreased gossypol. It would appear from these results that it is a common practice in the cottonseed oil mills visited to cook at low moisture levels in the cooker, since the subsequent drying operations would then be easier to perform. The moisture available in the cooker probably does not reach the gossypol glands, which are intact in the majority of material when it reaches the expeller. In this operation, where high temperature and pressures develop, the glands are broken and the gossypol flows out

TABLE II. Changes in Fat, Nitrogen, Free-Amino Groups of Lysine and Free and Total Gossypol During Cottonseed Processing

Position in Process[1]	Fat %	Nitrogen %	Lysine g/16gN	Gossypol (fat free basis) Free mg%	Gossypol (fat free basis) Total g%
			Press		
Seed	34.4	5.01	3.13	203	1.94
Cooker	28.0	4.34	3.22	193	1.48
Driers	29.6	4.00	3.52	200	1.46
Conditioner	30.4	4.38	3.12	200	1.49
Expeller	5.2	6.22	2.83	43	1.16
Product[2]	4.7	5.33	2.48	46	1.14
			Prepress-Solvent		
Seed	33.1	4.89	3.63	1820	1.82
Cooker	32.6	4.70	3.41	1630	1.77
Drier	34.2	4.64	3.79	1600	1.85
Conditioner	35.6	4.74	3.70	1550	1.89
Expeller	13.3	6.03	3.71	106	1.07
Tower	3.8	7.07	3.58	79	1.01
Product	3.3	8.20	3.50	81	1.07

[1] Samples taken after operation.

[2] Ground to pass 80% mesh.

into a product from which most of the oil has already been extracted. Since temperatures are high, 260-280°F, gossypol reacts with the protein, decreasing the available lysine concentration.

In collaboration with the engineer of the Borgonovo Plant in El Salvador, several factors were studied. The results are presented in Table III (11). When hulls are present free gossypol is lower and E-NH_2 lysine higher than when they are absent. Another finding of interest is that decreasing the load in the expeller results in a better quality product with respect to both available lysine and free gossypol content. From these findings it has been tentatively recommended that the following be taken into consideration for the production of cottonseed flour: 1) the use of good quality seed, 2) the use of the normal amount of hulls, 3) the use of moisture levels of 15-18% in the cooker, 4) the use of a lower load, 60-70% of normal, in the expeller. The use of hulls favors the production of a good flour probably by providing cushion action on the meal so that the oil flows better and to some exent protects the protein from heat destruction. If combined with the other suggested conditions gossypol will flow with the oil. From the experience gained it is evident and highly recommended that efforts should be made to carry out studies in cottonseed oil mills where the flour is the main product. The presence of hulls is not an obstacle in the production of a good quality flour, since they can be removed at the end of the process by a simple screening operation. The data given in Table IV (15) show that screening yields a product of higher quantity and quality of protein.

It would appear, therefore, that further improvement could be achieved by eliminating the very fine particles and using the coarser particles to give a higher yield of flour. Other results, which need confirmation, are those relating to the amount of water in the meal before entering the expeller and to the solvent extraction operation. Preliminary results suggest that the presence of small amounts of water in the meal before entering the expeller results in higher E-NH_2 lysine values. Likewise, small quantities of water in the meal before entering the solvent extraction tower has resulted in lower levels of free gossypol.

TABLE III. Changes in Free Gossypol on Free-E-Amino Groups of Lysine and During Processing of Cottonseed Under Different Conditions

Sample Taken	Normal[1]	75% Load	60% Load	Without Hulls	With Hulls
After	FREE GOSSYPOL, mg/100g				
Delinters	960	999	999	1250	900
Cooker	960	985	985	1170	940
Driers	940	970	970	1190	940
Conditioner	943	927	952	1047	923
Expeller	123	106	82	142	121
Tower (Final Product)[2]	91	58	47	92	68
E-NH_2-Lys, %	1.56	1.74	1.79	1.71	1.84

[1] Amount passed through expeller

[2] Ground to pass 80 mesh, at least 50% protein

TABLE IV. Effect of Screening a Pre-Press Solvent Extracted Cottonseed Meal on the Chemical Composition and Nutritive value of Several Granulometric Fractions

Analysis	Whole	Particle size, mesh coarser than					Finer than
	Meal	20	40	60	80	100	100
Ether Extract, %	3.4	3.0	3.3	3.8	4.0	4.1	4.0
Crude Fiber, %	9.6	19.7	9.5	5.6	5.2	4.6	3.5
Nitrogen, %	6.19	4.71	6.48	7.42	7.38	7.50	8.02
Free Gossypol, mg/100g	64	55	60	64	63	60	71
Total Gossypol, %	0.89	0.63	0.85	0.95	0.95	1.00	1.09
E-NH_2 Lysine, %	1.50	1.35	1.94	2.18	1.99	2.18	2.16
Weight gain[1], g	106	113	128	108	112	109	98
PER[2]	2.16	2.00	2.21	2.13	2.20	2.14	2.05

[1] Experimental period 28 days, initial weight 52 g.

[2] Protein Efficiency Ratio, weight gain/protein consumed.

Biological Studies with Chicks

The cottonseed meal producers in the Central American area have expressed interest in using high quality cottonseed flour in broiler rations since other concentrates such as soybean meal must be imported at a high cost. Therefore, studies were carried out to determine the nutritive value and amino deficiencies of cottonseed flour for the baby chicks (16). The results shown in Fig. 1 indicate that chicks can be fed up to 56% cottonseed flour containing 0.045 mg/100 g of free gossypol and 3.5 g lysine/ 16 g. Higher amounts decreased growth of the chicks although it may not have been due to higher gossypol intakes. This is postulated in view of the findings of Hale and Lyman (17) who reported that a higher protein level of intake increased gossypol tolerance in swine. The figure also shows the effect of supplementing 40% cottonseed flour diets with lysine, which improved growth significantly but not above the growth obtained with 56% cottonseed flour. This finding suggested that other amino acids became limiting. Further investigations suggested that threonine in the presence of lysine resulted in improved growth and methionine in the presence of the two improved growth still further. In another study summarized in Fig. 2, protein intake in the diet was increased with higher levels of cottonseed flour and each level was supplemented with lysine to give a constant total lysine to protein ratio in the diet 0.68. It can be seen again that 56% cottonseed flour gave highest growth, while higher levels decreased growth. Lysine addition at each protein level improved growth only in diets containing 40 and 48% cottonseed flour. Hemoglobin concentration was not improved by either protein intake or lysine level and was below that of a control group. Total serum protein and albumin paralleled growth response obtained with higher levels of protein intake; however, lysine addition at each protein level did not bring about an increase in total serum protein or albumin.

Studies of other investigators and reviews on the subject of gossypol have suggested that the protecting effect of higher levels of protein on gossypol was probably due to higher levels of lysine. Since chicks are very sensitive to lysine supplementation as to lysine deficient diets, it was of interest to learn whether or not lysine supplementation would counteract the action of gossypol. The results given in Fig. 3 were obtained in one case by feeding

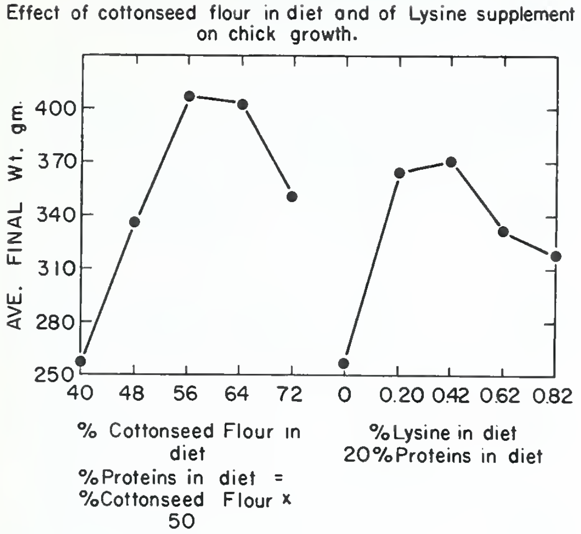

Figure 1

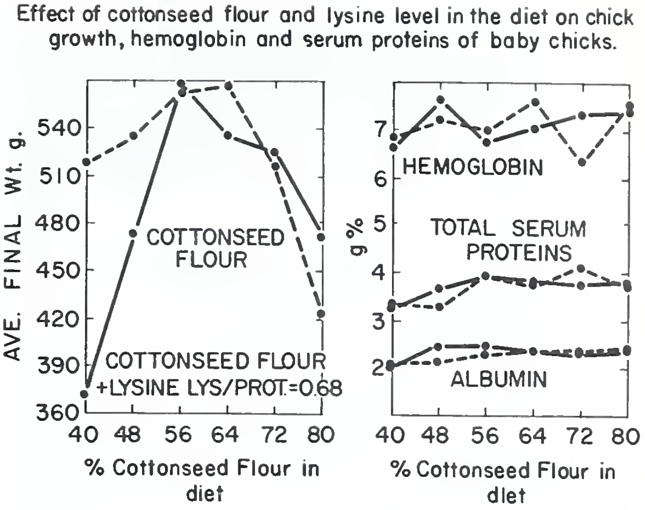

Figure 2

40% cottonseed flour with three lysine supplements 0, 0.2 and 0.5% and with 5 levels of gossypol added to the diet, 20, 40, 60, 80, and 100 mg per 100 g. During the first 3 weeks, lysine improved growth, but higher gossypol levels had no effect on growth at any level of lysine. During the last 3 weeks crystalline gossypol was replaced by an amount of ground cottonseed which would provide the same amounts of free gossypol. It can be seen that as the amount of ground crude cottonseed increased, growth decreased at all levels of lysine supplementation. These results were confirmed in other experiments with lysine supplementation as high as 0.75% of the diet. It would seem, therefore, that the depression ingrowth could be due to two possible causes. Either cottonseed contains a toxic factor different from gossypol, or the gossypol in the gossypol glands or in the raw cottonseed is much more effective than free gossypol in causing adverse physiological effects. Finally, lysine supplementation did not protect the chick against the adverse effects caused by substances present in raw cottonseed.

Biological Studies with Rats

These studies were conducted in order to obtain more information on the factors which affect the nutritive value of cottonseed meal and flour. Eighteen samples of cottonseed oil meals from different countries in Central America were analyzed for their proximate composition as well as for their free and total gossypol, E-amino lysine, total lysine, methionine, and threonine content (18). Biological tests were also performed by feeding the different samples at a protein level of 10% in the diet to 8 female and 8 male weanling rats for a period of 8 weeks.

Table V summarizes the analytical and rat growth data of all 18 samples. Ether extract ranged from 0.7 to 10.4 g/100 g with an average of 5.2, crude protein ranged from 40.0 to 53.0 g/100 g with an average of 47.2, E-amino lysine ranged from 1.39 to 2.22 g/100 g with an average of 1.78% or 3.11 to 4.38 g/16 g N with an average of 3.78, free gossypol ranged from 40.1 to 128.2 mg/100 g with an average of 70.7, and total gossypol ranged from 0.937 to 1.264 g/100 g with an average of 1.069 g. The lysine content ranged from 2.08 to 2.90, with an average of 2.45; methionine from 0.46 to 0.76 g/100 g with an average of 0.66 and threonine from 1.51 to 1.92 with an average of 1.70 g. (18, 25).

From the analytical data and the results of the biological test (18, 25), several re-

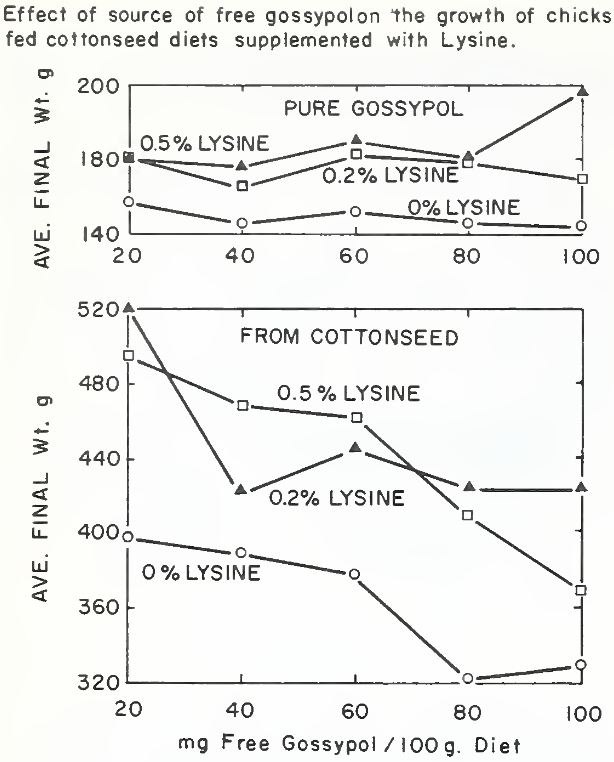

Figure 3

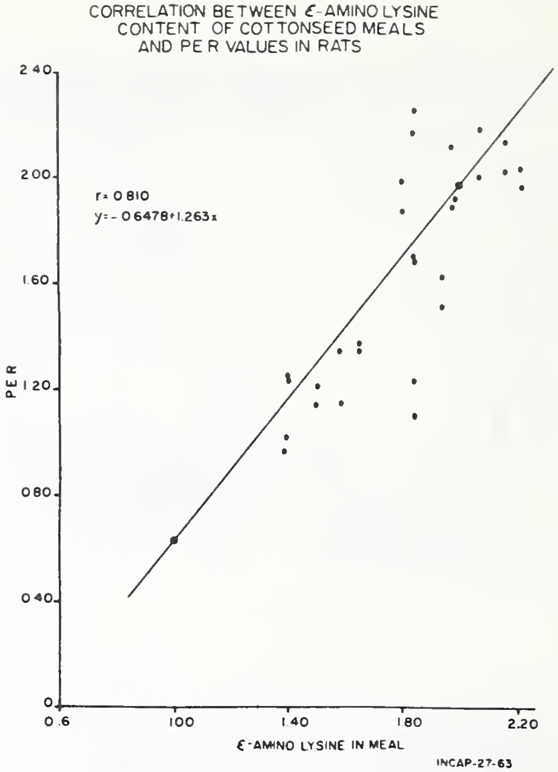

Figure 4

lationships were established as shown in Fig. 4. There was a highly significant correlation of 0.81 between E-amino lysine and PER. This was expected since cottonseed protein is limiting in lysine, and a better PER would result from higher lysine concentrations. Fig. 5 shows a significant negative correlation of -0.68 between weight gain and total gossypol. This is not surprising since the relationship is really between bound gossypol and weight gain, the amounts of free gossypol being very small in com-

TABLE V. Partial Chemical Composition, Rat Growth and PER of 18 Cottonseed Flour Samples from Cottonseed Mills in Central America

Substance	Range	Ave. S. D.	Substance	Range	Ave. S.D.
Ether Extract %	0.7 - 10.4	5.2 ± 2.8	Protein, %	40.0 -52.8	47.2 ±4.4
Free Gossypol mg/100g	40.1 -128.2	70.7 ±22.5	$E-NH_2$-Lys g/16 g N	3.11+ 4.38	3.78±0.41
Total Gossypol	0.94- 1.26	1.70± 0.15	Crude Fiber, %	3.2 - 9.9	5.6 ±2.6

Biological Test

	Weight Gain, g	PER
Males	34-114, 74	1.11-2.25, 1.07
Females	34-101, 73	0.96-2.16, 1.68

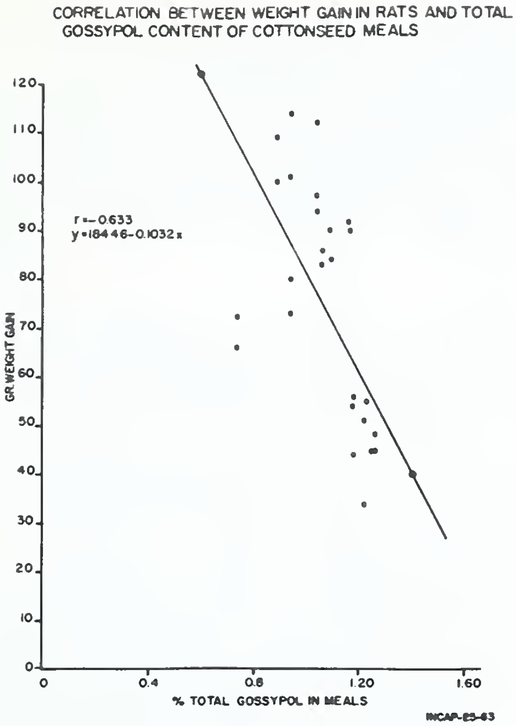

Figure 5

parison to total gossypol. More bound gossypol means less available lysine and thus, less growth. Fig. 6 illustrates the correlation between the residual fat content in the meals and PER. These two variables correlated negatively with a coefficient of -0.62 which was significant at the one percent level. A negative relationship Fig. 7(r=-0.77) was also found between the residual fat content in the meals and weight gain. These last two relationships have not been reported previously for cottonseed flour or meals and it appears that residual fat is an additional factor affecting the nutritive value of cottonseed flours.

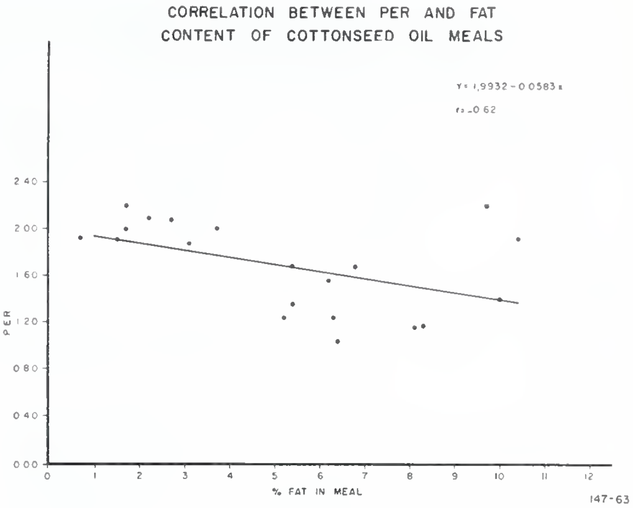

Figure 6

In a further series of experiments poor quality meals were found to have greater amounts of oils which did not react with urea and which were shown to be toxic to experimental animals. Iodine numbers were higher for oils not reacting with urea than those forming urea adducts. Other biological tests carried out with rats indicated that poor quality meals even when supplemented with lysine to give a high level of available lysine equal to that of good quality meals, produce growth with a PER much lower than that from good quality meals. This suggested that other amino acids in addition to lysine had been damaged during processing. Further work is being carried out on the residual fat of the meals to find out if and how it interacts with protein quality and gossypol. Studies carried out in INCAP (11) with swine fed diets adequate in all nutrients but with different levels of cottonseed meal have demonstrated that as the level of cottonseed meal in the rations increased, the animals showed the following symptoms which progressively worsened with time: diarrhea, rough hair coat and skin lesions. The latter have not been reported as symptoms of gossypol toxicity but rather as a result of a low fat intake and especially as a deficiency of essential fatty acids.

The Effect of Cooking on the Gossypol Content of VM 9 and on its Nutritive Value in Dogs

At the Conference in Cottonseed Protein for Animal and Man held in November 1960 in New Orleans, it was reported that cooking Vegetable Mixture 9 (VM9) with large amounts of water as is done in preparation for human consumption, decreased free gossypol and

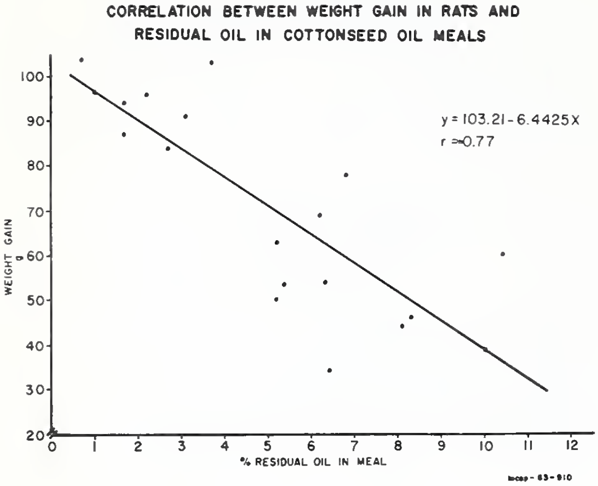

Figure 7

did not change in E-amino lysine (13). Further work on this problem has been carried out since and Table VI summarizes the results of several experiments (19). The Table shows the effect of cooking alone and in the presence of calcium and ferrous ions on the free and total gossypol content of samples of Vegetable Mixture 9.

Cottonseed flours containing from 32 to 96 mg of free gossypol were used in the preparation of the mixtures for study. For cooking the ratio of Vegetable Mixture 9 to water was 1 to 5.

It can be seen that in this experiment also a decrease in free gossypol takes place when the mixture is cooked for 25 minutes (11). Calcium ion decreases free gossypol further and the presence of ferrous ion with calcium decreases not only free gossypol but total gossypol as well. Ferrous ion alone does not seem to decrease free gossypol. Table VII shows that a decrease in gossypol takes place merely by wetting the food mixture containing cottonseed. These data represent the average of 8 to 12 analyses of samples of Vegetable Mixture 9 made from cottonseed flours with different free gossypol content. Wetting in the alkaline state decreases free gossypol significantly and a further significant decrease results when

TABLE VI. Effect of Cooking on the Free and Total Gossypol Content of Vegetable Mixture 9 in the Absence and Presence of Calcium and Ferrous Ions

Addition to Vegetable Mixture 9[1]	Free Gossypol in Cottonseed Flour[2]	Gossypol					
		Free (mg/100g)			Total (g/100g)		
	mg/100g	Initial	Final	% Loss	Initial	Final	% Loss
None	32-84	22.4	10.8	51.7	0.34	0.35	-
$CaCO_3$	45-60	17.9	7.6	57.5	0.41	0.40	-
$CaCO_3$ +Sugar	45	12.5	7.1	43.2	0.26	0.27	-
$Ca(OH)_2$	32-96	17.9	5.5	69.2	0.33	0.33	-
$FeSO_4$	32-96	16.8	10.1	40.0	0.34	0.33	2.9
$CaCO_3$ +$FeSO_4$ +Sugar	45	4.6	2.6	43.5	0.27	0.26	3.7
$FeSO_4$, pH 9.15	84	22.1	0	100.0	0.41	0.38	7.3
None, pH 9.18	84	31.5	3.1	90.1	0.43	0.42	2.3
$Ca(OH)_2$ +$FeSO_4$	32-96	10.9	2.9	73.4	0.31	0.25	19.3

[1] 1% $CaCO_3$, 1% $Ca(OH)_2$, 0.1% $FeSO_4$, 40-50% Sucrose

[2] Quantity used in Vegetable Mixture 9 - 38%

TABLE VII. Effect of Water Addition, Calcium Hydroxide and Ferrous Sulfate to a Cottonseed Flour Vegetable Mixture on Its Free and Total Gossypol Content.

Treatment or Vegetable Mixture 9	Free Gossypol[1]			Total Gossypol[1]		
	Untreated	Soaked	Decrease %	Untreated	Soaked	Decrease %
None	24.8	22.2	10.5	384	381	-
Alkaline	21.4	14.9	30.4	370	368	-
$FeSO_4$	18.8	13.9	26.1	332	355	-
Alkaline +$FeSO_4$	14.5	3.5	75.8	328	257	21.6

[1] mg/100 g, dry weight basis. All analyses were performed on freeze dried samples. The values represent the average from 8 to 12 observations.

the mixture contains calcium hydroxide and ferrous sulfate (19).

The mechanism of the disappearance or destruction of gossypol upon wetting and cooking with calcium and ferrous ions is still not understood. A possible explanation is that the pigment or pigments are converted to compounds such as gossypurpurin which later decompose due to the conditions of the treatment applied. Lysine is not affected, however, since available lysine and the nutritive value of the protein of the cooked mixture are as high as when cooked for less time or when fed raw. It can, therefore, be assumed that the decrease in gossypol takes place in a different manner than when gossypol is decreased during cottonseed processing for oil extraction.

Representative results (19) showing that available lysine is not affected upon cooking are given in Table VIII. The lower values for available lysine were obtained when sugar was added and are a result of dilution. The fact that the nutritive value is not affected is shown in Table IX. Three dogs were fed Vegetable Mixture 9 cooked for 0, 8, 16, and 24 minutes. Feces and urine were collected for nitrogen balance determinations. Retention of nitrogen remained high and equal for all cooking times. If lysine had been made unavailable by cooking, nitrogen retention would have decreased. Experiments are now underway to study the mechanism by which gossypol disappears upon cooking.

Studies with Dogs

In order to learn more about the fate of gossypol ingested by the animal fed Vegetable Mixture 9, studies were carried out to determine if the pigment was present in the feces (20,21). The fecal material used was collected for the studies described above on the effect of cooking on the nutritive value of the protein of Vegetable Mixture 9. Representative samples of the food cooked for several periods of time and the individual seven-day collections of feces from each of three dogs was freeze dried and then analyzed for free and total gossypol by means of the reaction with aniline. From the total intakes and excretions, gossypol balances were calculated.

The results indicate that all total gossypol fed appeared in the feces, while free gossypol in the feces was about 3.4 times higher than that ingested (Table X). Since total gossypol includes free gossypol and good recoveries were obtained for total gossypol in the feces, it would seem that the higher fecal free gossypol was due to liberation of the bound gossypol in the intestinal tract. The possibility existed, however, that free gossypol values in the feces were high because the method was measuring gossypol plus other fecal pigments.

Enough fecal material from several dogs fed Vegetable Mixture 9 was collected and freeze dried. The dried material was extracted with 70% acetone or with acid chloroform and maximum absorption curves of the original extract, of chromatographic fractions of the original extract and of the dianilido gossypol and phloroglucinol derivatives were carried out and compared to pure gossypol and derivatives of pure gossypol. Table XI summarizes the results. It can be seen that the pigment present in the feces was gossypol.

TABLE VIII. Effect of Cooking Vegetable Mixture 9 on Free and Total Gossypol and Free-NH_2 Groups of Lsyine Content

Addition to Veg. Mixt. 9	Gossypol analysis mg/100g						
		0	5	10	15	20	25
+Sugar+0.2% enzyme 1/	Free	12.5	11.9	8.1	7.1	7.3	7.1
	Total	262	305	394	254	267	267
	E-NH_2-Lys	658	676	658	664	682	626
+Sugar+0.2% enzyme +0.1%$FeSO_4$ 1/	Free	4.6	4.0	2.6	4.0	3.2	2.6
	Total	275	266	255	234	266	258
	E-NH_2-Lys	626	664	684	658	696	746
Cottonseed flour alone	Free	46.3	41.5	36.1	33.1	33.0	27.5
	Total	941	905	947	909	951	858
	E-NH_2-Lys	1558	1529	1570	1470	1448	1547
+0.24%Lys+0.2% enzyme 2/	Free	16.7	15.7	11.3	9.8	10.1	9.7
	Total	411	422	378	381	390	380
	E-NH_2-Lys	932	911	900	888	862	944
None 3/	Free	33.3			15.4		13.9
	Total	469			399		432
	E-NH_2-Lys	1049			1037		1098
+0.1% $FeSO_4$ 3/	Free	25.8			19.7		17.5
	Total	410			421		404
	E-NH_2-Lys	1025			944		1019

1/ Veg. Mixt. 9 contains 1% $CaCO_3$; free gossypol in flour 45 mg/100g

2/ Veg. Mixt. 9 made with cottonseed flour shown above. Formula contains 1% $CaCO_3$

3/ Veg. Mixt. 9 contains 1% starch instead of 1% $CaCO_3$ or 1% $Ca(OH)_2$. Free gossypol in flour 96 mg/100g

TABLE IX. Nitrogen Balance of Dogs Fed Vegetable Mixture 9 Cooked for Several Periods of Time. (Ave. 3 Dogs)

Nitrogen	Cooking Time, min.				
MgN/Kg/day	0	8	16	24	30
Intake	844	862	862	865	854
Feces	234	241	249	253	257
Urine	462	481	472	471	481
Absorbed	610	621	613	612	597
Retained	148	140	141	141	116
Absorbed**	72.3	72.0	71.1	70.7	69.9
Retained **	17.5	16.2	16.4	16.3	13.6

** Percentage of Intake

TABLE X. Gossypol Intake and Fecal Excretion of Dogs Fed a Cottonseed-Corn Mixture Cooked For Several Periods of Time

	Cooking, min.				
Gossypol	0	8	16	24	0
Intake					
Free (A)	31	30	26	22	34
Total	709	689	704	715	780
Fecal					
Free (B)	108	91	86	83	103
Total	703	687	757	735	805
Free B/A	3.5	3.0	3.3	3.8	3.0

Average 3 Dogs
mg/dog/day
7 days/cooking period

TABLE XI. Wave Length of Maximum Absorption of Gossypol and Extracts From Feces of Dogs Fed Cottonseed Flour in Vegetable Mixture 9, and of Their Aniline and Phloroglucinol Derivatives.

Material	Reaction	Max. Abs.
70% Acetone-H_2O Extract of feces	None	370-380 mμ
Gossypol	None	370-380 mμ
70% Acetone-H_2O Extract of feces	Aniline	440 mμ
Gossypol	Aniline	440 mμ
70% Acetone-H_2O Extract of feces	Phloroglucinol	550 mμ
Gossypol	Phloroglucinol	550 mμ
Chromatographed fecal Chloroform extract	Aniline	440 mμ
Gossypol	Aniline	440 mμ
Chromatographed fecal Chloroform extract	None	370-380 mμ
Gossypol	None	370-380 mμ

Figure 8 presented as an example, shows the absorption curves of the feces extract chromatographed on florisil, eluted with different solvents and reacted with aniline. All the solutions studied gave a maximum absorption peak of 440 mu. Other tests carried out are in Table XII. All gave positive results for gossypol, including the egg yolk discoloration test. This test was carried out with four hens per group and eggs collected for 10 days. The hens were fed a diet which did not contain any cottonseed meal, but to which the following were added: dried feces, fecal extract, the chromatographic fractions showing higher gossypol content, pure gossypol and cottonseed flour.

Other experiments were then carried out in dogs to study the factors affecting gossypol absorption since it was known that gossypol had been isolated from pig livers by Smith (22, 23). However, level of calories, different types of fat in the diet as well as different methods of cooking the mixture did not change the results found previously, gossypol was being excreted in the fecal material as before. In subsequent investigations dogs were fed the Vegetable Mixture to which gossypol was added, either as the pure substance or as found in crude cottonseed oil. In these cases, too, gossypol was recovered in the feces. In still other experiments, the results of which are shown in Fig. 9, dogs were fed a casein diet, followed by the casein plus gossypol diet and finally the casein diet. Daily collection of feces was carried out. As can be seen, when no gossypol was fed, no gossypol was found in the feces. The graph shows a peak excretion of gossypol. It was also found that better recoveries of gossypol are obtained from seven day balances than from balances of four days' duration.

Two possible explanations can be offered according to the evidence available. It is possible that when gossypol is present in the diet in relatively small amounts, none is absorbed. However, if any is absorbed, it is secreted back into the intestine, possibly via the bile duct. The latter is now being studied in dogs and swine. Studies

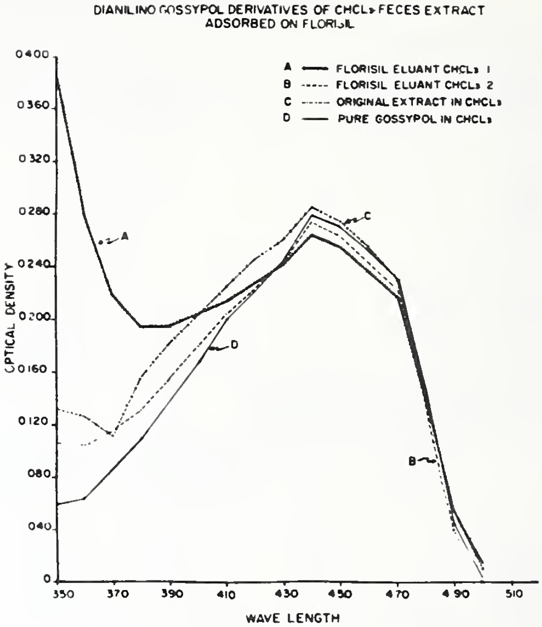

Figure 8

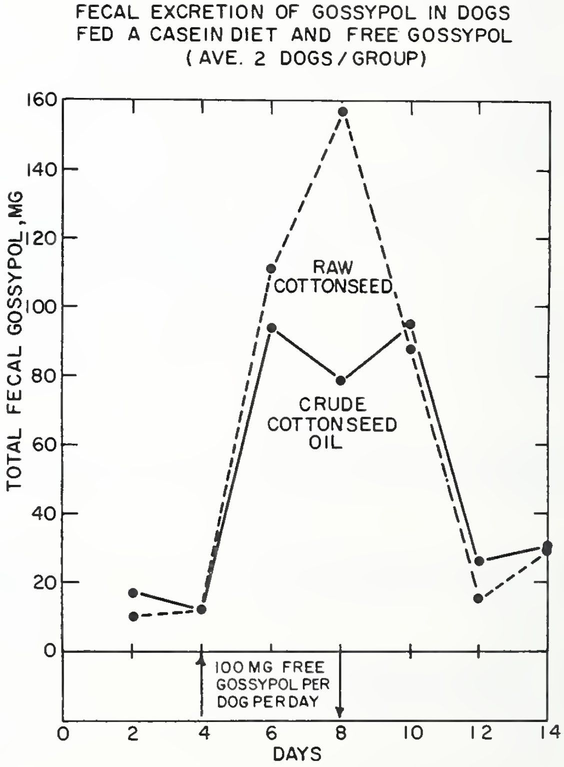

Figure 9

TABLE XII. Qualitative Reactions on Feces and Fecal Extracts of Dogs Fed Cottonseed Flour in Vegetable Mixture 9.

Test	Reaction
Fecal Extract	
+NaOH+H_2O_2	+
+Aniline	+
+Phloroglucinol	+
+$SbCL_3$	+
Fed to hens	Egg Yolk discoloration
Feces fed to hens	Egg Yolk discoloration

were extended to children and gossypol was found in the fecal material. The pigment disappeared from the feces when the children were no longer fed the mixture. The results are, however, not as clear-cut as with the dogs.

Studies with Swine

Studies are being performed at INCAP with swine, for the purpose of a) obtaining additional information on the pathology of gossypol toxicity, b) confirming biologically the observations made when the Vegetable Mixture was treated with calcium and ferrous ions, and c) learning if gossypol is being excreted with the fecal material (24). Three Duroc Jersey pigs of 8-9 weeks of age per group were used and fed one of five diets. Each feeding trial was repeated 3 times making a total of 9 pigs per diet group.

In all trials, the animals were fed individually a diet containing 42% cottonseed flour which contained 43% protein, 3.39 g/16gN E-NH_2 lysine, 93 mg of free gossypol per 100 g and 0.91% total gossypol. The protein content of the diets as fed was 24.4, while that of the control group not receiving any cottonseed meal was 27.6%. The diets were analyzed for free gossypol after mixing and before feeding. The other ingredients of the diet as fed were 3.0% salt mixture, 3% alfalfa leaf meal, 51.8% corn and 0.2% aurofac.

A partial summary of the results of the one replication so far completed is presented in Table XIII. It can be seen that mortality, which occurred between the 7th and 12th week after initiation of the test, while cooking with water in the ratio of solids to liquids of 1/3, had 60% protection. The addition of 1% $Ca(OH)_2$ and 0.1% $FeSO_4$ to the diet had 100% protection whether the diet was fed raw or cooked with relatively large amounts of water. The pigs consuming 42% cottonseed with supplements of $Ca(OH)_2$ and $FeSO_4$ also showed hemoglobin and hematocrit values similar to those of the control group. These results, therefore, confirm the analytical findings presented earlier as well as previous findings with swine fed Vegetable Mixture 9 (4, 11). The other replicates gave similar results, except that cooking alone has indicated a lower protection to the animal than the one indicated in the results shown above.

Summary

The results summarize part of the work carried out in INCAP since 1960 on several technological aspects of Vegetable Mixture 9 (Incaparina). Studies on the effect of variety and on environment on the chemical composition of cottonseed, lysine and gossypol content indicate that both genetic composition and environment affect the concentration of fat, protein, lysine, and gossypol in the seed, while crude fiber and ash are little affected.

The results of experiments performed in cottonseed mills using the pressing process and the prepress solvent extraction process indicate that the losses in lysine occur in the expeller operation, where high temperatures develop. From trial runs in the prepress solvent mill, it was found that the presence of hulls results in a flour with lower free gossypol content and higher available lysine. Decreasing the load in the expeller also resulted in cottonseed flour of higher quality. It was found that the moisture level at the cooking step was too low to rupture the gossypol glands, and from laboratory and mill studies the following recommendations were made: 1) to use a moisture level in the cooker of 15-18%, 2) to use hulls, and 3) to decrease the load in the expeller to about 70% of normal.

Further studies indicated that very fine cottonseed flour particles, representing

TABLE XIII. Effect of Cooking and of the Addition of Calcium Hydroxide and Ferrous Sulfate to 42% Cottonseed Meal Rations on the Performance of Growing Swine.

Diet Fed	Free Gossypol Weight, lbs. in Diet[1] mg/100 g.				Feed Efficiency [2]	Mortality	Hemoglobin % Hematocrit			
	Before	After	Initial	Final			Initial	9 wks	Initial	9 wks
Control	0	0	22.2	89.0	3.3	0/3	13.6	13.7	42.3	42.7
Untreated Cottonseed meal[3]	40	27	22.0	27.5	9.6	3/3	11.9	8.5	37.7	24.0
Cooked Cottonseed meal[4]	42	17	21.7	65.2	3.8	1/3	11.7	10.4	37.7	31.0
Untreated Cottonseed meal + 1% $Ca(OH)_2$ + 1% $FeSO_4$	26	12	21.7	82.3	3.6	0/3	11.6	14.0	37.7	39.7
Cooked Cottonseed meal +1% $Ca(OH)_2$ + 0.1% $FeSO_4$	44	10	21.7	79.3	3.7	0/3	12.3	14.5	39.0	40.3

[1] Content before and after treatment.
[2] Ave. feed consumed/Ave. weight gained.
[3] Water added but no heat applied.
[4] Water added, heat applied.

about 8% of the total contain large amounts of free gossypol, and by a simple screening operation the quality of the flour could be upgraded. In biological tests with chicks, it was found that good growth could be obtained with up to 56% cottonseed flour, but higher levels depress growth. Lysine addition to a 40% cottonseed flour diet improves growth but not to the level obtained with 56% flour. Probably, the next limiting amino acids are threonine and methionine.

Addition of up to 100 mg of free gossypol to chick diets containing 40% cottonseed flour and several levels of lysine did not affect growth or decrease the effect of lysine. However, addition of a similar amount of gossypol from raw cottonseed depressed growth even when the diet was supplemented with 0.75% lysine. This suggested that either gossypol in the gland is more toxic than crystalline gossypol or that cottonseed contains other toxic factors.

In tests with rats highly significant correlations were found between PER and available lysine (r=+0.81), between weight gain and total gossypol (r=-0.63), between residual fat in the meal and growth or PER (r=-0.77, r=-0.62). These results further demonstrate that residual fat is another factor to consider in the evaluation of the nutritive value of cottonseed flour.

Studies on the effect of wetting and cooking on the gossypol and available lysine content of Vegetable Mixture 9 without and with calcium hydroxide and ferrous sulfate alone or combined were carried out. Wetting alone without salts caused a 10% decrease in free gossypol. When Vegetable Mixture 9 contains $Ca(OH)_2$ a 30% decrease is obtained, when $FeSO_4$ is present alone a 26% decrease is observed and in the presence of both, there was a 76% decrease in free gossypol. Cooking further decreases both free and total gossypol while there is no change in available lysine. This was also shown in nitrogen balance experiments with dogs.

In dogs 100% of the gossypol intake could be recovered in the feces. The gossypol was isolated from the feces and identified as the pure compound and as the dianilido and pholroglucinol derivatives. Biological tests with hens also indicated the compound isolated from the feces to be gossypol. Several factors were studied to determine if they affected gossypol absorption. Calories from CHO or fat, type of fat, or cooking method did not affect gossypol excretion and in all cases 100% recoveries were obtained.

Studies with swine were also carried ou to test biologically the effects of wetting and cooking of Vegetable Mixture 9 on free gossypol. Swine were fed 42% cottonseed flour with a free gossypol content of 92 mg/100 g. The results indicated that feeding untreated cottonseed flour resulted in 100% mortality of swine; cooking reduced mortality to 60% while addition of calcium hydroxide and ferrous sulfate, with or without cooking, afforded 100% protection to the animals. In these cases growth of the pigs was comparable to that of the control group and hemoglobin, hematocrit and serum protein values did not differ from those found for the control pigs.

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DISCUSSION

N. S. Scrimshaw: In previous meetings of this type, one of them at least here in New Orleans, a great many questions were asked about the effect of cooking on gossypol, the reaction between gossypol and lysine and the many other aspects of factors influencing the nutritional value of cottonseed meal. In the paper which Dr. Bressani has given we are beginning to get specific data and some concrete answers to some of the problems. I would like to ask Dr. Bressani to comment on some additional information which he either didn't have time to present or which has only become available since he prepared his paper. One question is the extent to which gossypol has been found in the liver and confirmed to be gossypol. A second is his opinion of recent results of INCAP studies on children in which both he and I have been collaborating.

Last July, four children were given gossypol at a moderate level in the form in which it occurs naturally in cottonseed flour. In the children, all aged from 7 1/2 to 12 years, the amount of free gossypol taken in and the amount excreted was essentially identical. However, taking all of the children combined, the total amount taken in was 33,000 milligrams and the total gossypol detected in the feces was only 25,000 milligrams.

A second series was then done with six children under rigid metabolic conditions, including a complete collection of feces and urine. In this study, with younger children, there was more free gossypol detected in the fecal secretion than in the intake. There was an even greater discrepancy between the total gossypol intake and the amount which could be detected in the feces. It should also be added that there was no indication of any relation whatsoever between the amount of the discrepancy in a given case and the level of gossypol intake using two different types of flours. Nitrogen balance was completely unaffected by differences in gossypol intake.

Ricardo Bressani: Concerning your question on the gossypol values in liver, I can only say that for the present, one must accept them as representing gossypol. Dr. Smith from North Carolina has been able to isolate and identify gossypol from the liver of swine fed cottonseed flour. Therefore, it is assumed that the substance extracted from liver with 70 percent aqueous acetone and reacted with aniline, is gossypol.

I would also like to add that a method different in certain steps from the one commonly used for cottonseed samples should be developed for the analysis of gossypol in animal tissue. The reason for this statement is that it is difficult to explain the observation made in our laboratory, but not reported here, that the liver of some rats fed cottonseed flour as the sole source of protein in the diet, sometimes contain gossypol while the liver of other animals on the same group does not. Likewise, the liver of rats fed cottonseed free and gossypol free diets shows gossypol values upon analyses.

As far as the gossypol values in feces are concerned, the evidence which we have accumulated, both chemical and biological, indicates that the material reacting with aniline is gossypol.

I wonder if I could have slide number 17 back on the screen; I think it is pertinent to this question concerning gossypol excretion in feces. In this study four dogs were used, divided into two groups. One group was fed gossypol as found in crude cottonseed oil and the other gossypol as found in raw cottonseed. Throughout the study a casein diet was fed. During the first four days the animals received no gossypol, during the second four days the dogs received 100 mg of gossypol per dog per day, and during the last period no gossypol was fed. The fecal material collected during the first four days apparently contained around 20 mg of gossypol. When gossypol was fed, significant amounts of gossypol were found in feces; there was a peak excretion followed by a constant decrease when gossypol was withdrawn from the diet. The values obtained during the third period were relatively low, but not as low as for those found in the first four day period. Only one dog per group gave zero values after gossypol was withdrawn. Total gossypol recovery was approximately 85 percent and gossypol was being excreted at the end of the study. These data indicate a delayed excretion.

Reviewing the history of these dogs, we found we had used them before in studies concerning the effect of cooking and other factors on the absorption of gossypol. In view of the results shown in slide 17, we concluded that the gossypol values found before gossypol was fed represented delayed excretion of the gossypol ingested in previous studies. Therefore we postulated that some gossypol was absorbed, stayed in the body for a while and then was excreted. In our next experiment we cannulated two dogs to collect bile; the dogs died because the cannula became separated from the bile duct. Analysis on the liver and bile indicated that these tissues contained gossypol. The conclusion was that some ingested gossypol was absorbed, transported to the liver, and secreted into the small intestine with the bile pigments. This would account for a delayed excretion. We are now working on the problem with more detail, particularly in trying to identify gossypol in bile, which seems to be an extremely difficult job.

N. S. Scrimshaw: So this is delayed excretion, you feel?

Ricardo Bressani: Yes.

N. S. Scrimshaw: Greatly delayed excretion?

Ricardo Bressani: It is difficult at this moment to say how delayed is the excretion of ingested gossypol.

N. S. Scrimshaw: And what are your speculations as to why in experiments run for three-day periods with children, such a relatively small percent of the total gossypol ingested is recovered?

Ricardo Bressani: I really do not know how to explain the results in children. In some cases the recoveries of ingested gossypol were good and in other cases they were not. It might be due to the manner in which the fecal samples were handled, which was different from the way the fecal material from the dogs was handled. These experiments should be carried out for a longer period of time. In any case, the average recovery in children is approximately 80 percent.

N. S. Scrimshaw: I think this is one of the unsolved problems that we must continue to study.

R. A. Phelps: Frank Smith has had a considerable amount of experience with detection of gossypol in various animal tissues, and I know Dr. Lyman has had some exchange of correspondence with Frank. Frank has amassed a considerable amount of data on gossypol deposition in liver, kidneys, heart, etc., of swine fed high levels of gossypol. When he tried to analyze the blood and the bile pigments for gossypol, he encountered the same kind of problem that Ricardo is talking about. He has changed to another solvent and has achieved considerable success. I think if you would write to Frank Smith he would help you out considerably with this problem.

N. S. Scrimshaw: Let's have Dr. Lyman's comments.

C. M. Lyman: I should like to comment primarily on the apparent secretion of more free gossypol than was fed. This is an artifact. When cottonseed meal is treated with proteolytic enzymes and the digest is analyzed by standard methods for the determination of free gossypol, a considerable increase in apparent free gossypol is found. In our experience, however, when we tried to identify the liberated gossypol chromatographically it wasn't there; what was there was a gossypol peptide. Now the confusing fact is that when we treat these gossypol peptide compounds with p-anisidine or with aniline we get a reaction; and so then when we examine the absorption spectra of the aniline derivative, sure enough, we have a perfect fit with what we get with gossypol. Indeed, what we have is the gossypol aniline compound, but this doesn't mean we had free gossypol. It means that there is an exchange during the reaction. This always takes place under the conditions where we have in any way solubilized the protein material by splitting it into fragments so that the gossypol complex is siluble in the solution to which aniline or p-anisidine is added.

N. S. Scrimshaw: Dr. Lyman, would you comment on the reverse finding indicated in the report of Dr. Bressani, a decrease in the free gossypol content of the cooked material without any decrease in the biological availability of lysine?

C. M. Lyman: Well now, I think we need to take into consideration the amount of gossypol which is disappearing and the amount of lysine that it takes to combine with this. When we reduce, for example, from 0.08 percent free gossypol down to 0.02 percent the actual amount of lysine that would be required to react with the gossypol is pretty small, and it might be rather difficult to measure the difference in biological value. I am sure that when we have iron present and probably when we have calcium salts, there are other mechanisms for decreasing or eliminating gossypol.

N. Subramanian: I would like to have some information on the quality of cottonseed meal used in the INCAP mixture. Is it merely screw pressed or prepress solvent extracted? Did the meal conform to the UNICEF specifications in all aspects? Secondly, you suggested cooking of the meal with calcium hydroxide and iron salts to reduce the gossypol content. Does the cooking bring about any marked change in the color of the food? Thirdly, are you completely satisfied with the method of estimation of free gossypol in the fecal matter? I believe this question has been partly answered.

N. S. Scrimshaw: Dr. Bressani, will you comment on the quality of cottonseed flour used in Incaparina, the effect of iron on coloration, and the satisfaction with the determination of gossypol in these preparations?

Ricardo Bressani: The cottonseed flour used in the commercial preparation of VM9 (Incaparina) is produced by a prepress solvent extraction process, in the Borgonovo Cottonseed Oil Mill in Zacatecoluca, El Salvador. We have worked quite extensively at the plant to be able to find the optimum processing conditions for the production of a high quality cottonseed flour. I believe we already have more information than two or three years ago and are able to produce a flour of high quality. Some of the studies carried out were reported here in my presentation. I would like to make public the cooperation given to us by Don Antonio Borgonovo who permitted us to collect samples and change conditions as we required. I would also like to acknowledge the cooperation of Ing. Luis Tiraboschi, who worked with us in obtaining more information on how to produce a good quality flour.

Samples of the cottonseed flour to be used in the preparation of mixture 9 are always checked in the INCAP laboratory for free and total gossypol, total protein and available lysine. Sometimes the material is fed to rats and chicks for biological testing. The samples meet most of the specifications set by the conference in New Orleans in November 1960. Sometimes the amount of free gossypol runs a little higher, however, the cottonseed flour is used because of its high protein value as determined by available lysine determinations and biological trials. Furthermore, the mixture contains calcium carbonate or hydroxide, which we have shown, chemically and biologically to reduce the level of gossypol significantly, when the mixture is cooked before consumption.

N. S. Scrimshaw: Excuse me, I think you mean "running slightly over," rather than a "high gossypol content."

Ricardo Bressani: Yes. The samples run slightly higher in free gossypol. As I already mentioned, however, the presence of calcium hydroxide or carbonate and the process of cooking the mixture before consumption reduced free gossypol levels to very low and even zero values, without decreases in protein value, even when the cottonseed flour contains relatively high levels of free gossypol.

N. S. Scrimshaw: The specifications of the WHO/FAO/UNICEF protein advisory group?

Ricardo Bressani: Yes, slightly above the specification. This calls for a value of 45 mg free gossypol. Our samples vary from 50 to 70 mg of free gossypol per 100 mg, but then our available lysine values are higher than for example, Proflo. These values vary from 3.5 to 3.8 g/16 gN.

Upon the introduction in the processing of the modifications listed in my presentation we now accept about 80 percent of the samples for use in Incaparina productions, while two or three years ago we accepted only about 40 to 50 percent of the samples submitted for quality control.

N. S. Scrimshaw: The effect on color?

Ricardo Bressani: The color of the mixture becomes darker upon cooking in the presence of iron. The darkness of the color depends on the concentration of iron. When used in high amounts, as for the studies with swine reported, the mixture becomes dark, however, the animals did not seem to care, since they consumed all the food offered.

N. S. Scrimshaw: What about the mixture for human consumption?

Ricardo Bressani: As I mentioned previously, the presence of iron causes a darkening of the color, which depends on the concentration of iron. Therefore, we are trying to find which is the maximum concentration of iron needed to cause a reduction of free gossypol beyond that obtained from cooking and presence of calcium hydroxide or carbonate. Other iron salts are also being tested. In Guatemala, people use raw sugar cane to sweeten the Incaparina drink. This material contains relatively large amounts of iron. When this sugar is added to Incaparina, the color of the cooked product becomes dark. When more than 0.1 percent of iron is added, a very dark color is obtained even without cooking.

N. S. Scrimshaw: There is one fortunate thing in the use of this preparation in Central America, the formula already has calcium hydroxide and then the people add this crude brown sugar or panela with a high iron content and cook it for 25 minutes.

Ricardo Bressani: That is correct. I do want to mention another fact. We tried different calcium salts, such as the calcium phosphates, but none were very effective in decreasing the level of free gossypol. Calcium hydroxide was more effective than calcium carbonate.

The method for the determination of free gossypol, consisting of extraction with 70 percent aqueous acetone and reaction with aniline, is reliable in the sense that several analyses on the same sample give the same value. The method, however, is for gossypol and gossypol-like pigments. The latter may not have adverse physiological effects. The total gossypol method is more reliable than the free gossypol method. The total gossypol values have shown better correlations with biological values in rats than the free gossypol content.

I should like to make one comment in relation to Dr. Lyman's statement. This is in regard to the amounts of free gossypol in the feces of dogs we have studied. In tests with about 18 or 20 dogs with which we were trying to find the mechanisms of absorption and excretion of gossypol, it was always found that the free gossypol in the feces was higher than the intake, but total gossypol the same. It was interesting to find, however, that the ratio of free gossypol in the fecal material to the intake is very constant, around 3.4.

N. S. Scrimshaw: Three point four what?

Ricardo Bressani: This is the ratio of the free gossypol content of the feces to the intake of free gossypol. It is always very constant. It varies between 3.2 and 3.8 in different dogs and for long periods of time. Probably it indicates a constant breakdown or hydrolysis of some type of bound gossypol.

N. S. Scrimshaw: My introduction to Dr. Bressani and his remarks have given you some of the background for the development of the vegetable mixtures based on cottonseed flour as a primary protein source by the Institute of Nutrition of Central America and Panama. The first country into which such a mixture was introduced was not surprisingly the one in which INCAP is located, Guatemala. About two years ago, the product was introduced in Guatemala by local industrial interests without the kind of commercial build-up which is normal for the introduction of food products in the more developed countries. It had, however, the advantage of the presence of INCAP, the knowledge and interest of the medical profession, the public health workers, and the school teachers. Despite the lack of what would be considered adequate commercial promotion, it was accepted from the beginning, and the consumption of Incaparina has gradually increased and is still continuing to increase in Guatemala, although it falls far short of reaching the majority of people in the rural areas where it is most needed. We were anxious to see it introduced into a large area and an area of equally great need with the advantage of modern know-how in distribution and marketing. We are now going to hear from Dr. Bruins, the Manager of Nutrition and Biochemistry Research of the Quaker Oats Company, on the preliminary results of such an effort in Colombia.

INTRODUCTION OF PROTEIN-RICH FOOD PREPARATIONS IN COLOMBIA

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During the past fifteen years, it has become abundantly clear that malnutrition, and particularly protein malnutrition in young children, contributes greatly to mortality rates in under-developed areas of the world. In Colombia, the problem is similar to that which has been described in detail for other Latin American countries. Infant mortality rates are in excess of 100 per 1000 live births or about 4 times the comparable U. S. figure. In the 1-4 year age bracket, about 20 times as many children die as in the United States (1).

In the last decade, much research has been done on these problems and results have pointed the way for possible solutions. Improvement in the economic situation and development of animal agriculture are certainly the most permanent solutions but are terribly long range. Surely, immediate and direct action on such a poignant human problem is necessary.

Efforts to solve the problem so far have been largely governmental, and it now appears that private industry with established merchandising and distributing know-how must come into the picture in order to manufacture and sell the type of product(s) needed. About four years ago we began exploring this area to determine whether we could make a contribution. It was decided to make our first effort in Colombia, where we were already established with a plant and marketing organization, manufacturing and merchandising Incaparina under an agreement with the Instituto de Nutricion de Centro America y Panama (INCAP).

Before describing our marketing program for Incaparina, I want to discuss some of our original analysis of the situation, on which subsequent decisions have been based. First, it should be pointed out that the problem of kwashiorkor exists primarily in countries where corn, cassava, rice, and sugar are staple items in the diet. Figure 1, adapted from Sebrell, (2) illustrates the reason for this. In rating the adequacy of the protein supplied by these various carbohydrate sources, both quality and quantity of protein in relation to the caloric content is considered. Quite obviously, if a child is weaned to a corn-sugar diet between 9 months and 2 years of age, he will get less than half the protein he needs. In fact, such a diet will not supply adequate protein, unless supplemented, until about age 10. Sufficient protein will accompany the calories consumed as rice by age 6, and wheat by age 2. Hence, in a population subsisting largely on wheat, and rye and barley would be similar, one would not expect to find severe protein deficiency.

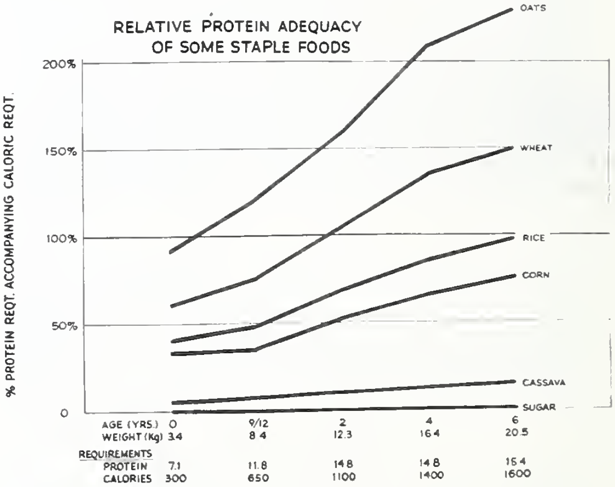


Figure 1

Oats and wheat are both well accepted by the Latin and if these could be used as staple cereals, particularly for youngsters, the protein problem might well be solved. However, oats has been widely distributed in Latin America for 45 years and obviously has not solved the problem--for the population as a whole. This simply points up the fact that technical adequacy, acceptance, and distribution are not sufficient criteria for the product needed.

Price of the product is also an important consideration, perhaps the most important one. In Figure 2 we have plotted data from a 1950 Costa Rican diet study (3), relating the total calories consumed of a particular product to the cost per unit of energy derived therefrom. It is apparent from these data that there is a close relationship between the cost of energy from a particular food product and the amount consumed. We believe that this relationship is a casual one.

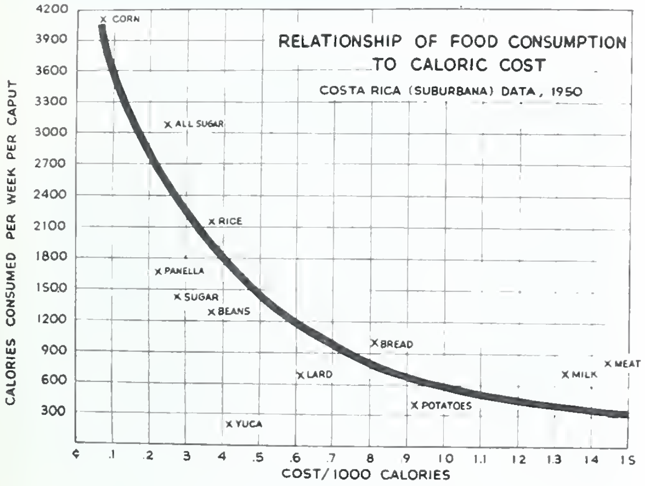


Figure 2

In contrast, Figure 3 depicts food consumption in the United States (4) on a similar basis, calculated on 1959-60 prices for those items used in the BLS food price index and weighted within classes in accordance therewith (5). Here, as one would have suspected, there is no apparent relationship between cost of calories and the amount consumed. Since the American consumer spends relatively little of his income for food, he has the option of buying what he likes and, perhaps, what he thinks is good for him.

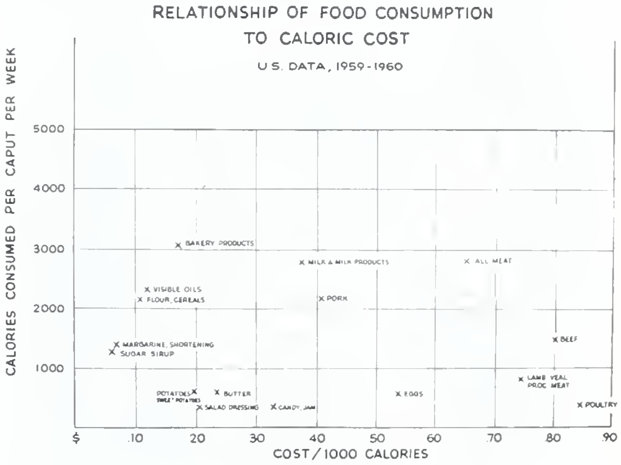


Figure 3.

The average, or lower class, Latin American, however, spends a high proportion of his income on food and thus, it appears, tends to buy that which supplies his caloric needs most economically. This suggests that education on other nutritive needs may ultimately build a demand for proteinaceous foods. Our survey interviews indicate, however, that this knowledge already exists, in a general way, but that economics presents too much of a hurdle.

Further, if we can accept the principle that man will buy first to satisfy his caloric needs and will purchase other nutritive needs only as he can afford them, then we can also reason that the further development of animal agriculture on the same kind of cost structure that now exists will not solve the problem unless the income of the people rises substantially, as well. It appears that it will be some time before these economies can afford the luxus animal protein diets which we enjoy. These considerations indicate that to get substantial market penetration of a product designed to supply protein needs, it will be necessary to have it priced in a range where it is competitive as an energy source with other staple foods.

Normally rice is consumed in good quantity in Latin American diets. Thus we have set the price of rice as a goal in establishing the price of a protein supplement.

The concept of the vegetable protein mixture fits into this problem especially well in Colombia. Because there is a relative scarcity of corn and rice, prices of cereals are high in relation to protein supplements. With cottonseed flour and soy flour both a little cheaper than corn, it is possible to price a protein-rich product competitively after manufacturing, packaging and other charges are assessed against the product.

Figure 4 shows the price relationship between Incaparina and other products--plantain flours, corn starch and dried milk--used in volume for feeding infants. In addition to being very competitive in price with these items, the $1.30 (Colombian pesos) per pound should also be compared with approximately the same prices for rice, and about $.90 for degerminated corn and panela (raw sugar). Thus we believe Incaparina is in a price range where substantial amounts can be sold even if people do not fully understand its nutritional benefits.

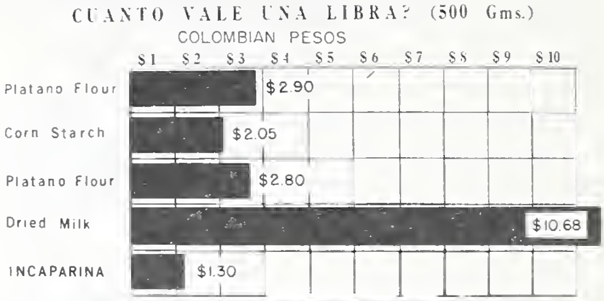


Figure 4. Comparative Costs

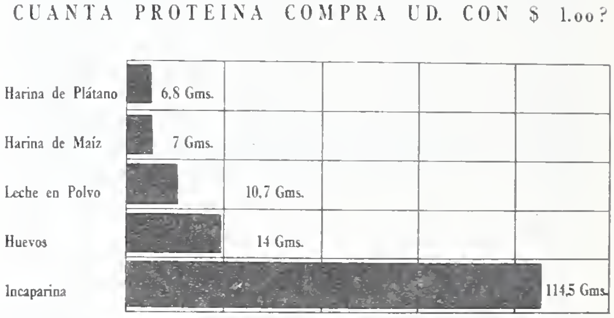


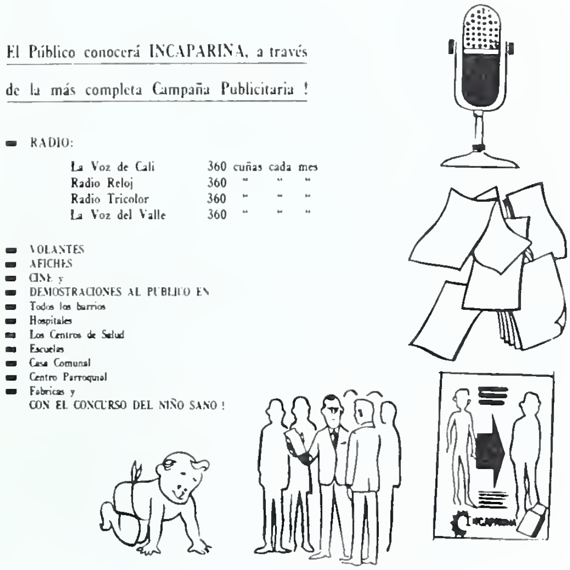
Figure 5

Figure 5 shows the same products figured in terms of the amount of protein supplied per unit of cost. This demonstrates the great economy of Incaparina in satisfying the protein needs of the population. Educating people to this need is a long range proposition but it is important if we are to achieve permanent marketing success.

The actual introduction of a protein food must be preceded by a program which will set the proper climate for success. We believe it is important that public health authorities and the medical profession in a country should be solidly behind a project that has such great promise in solving a key health problem. Dr. Rueda Williamson of the National Institute of Nutrition, Dr. Baquero and Peidrahita of the Ministry of Health, Dr. Sinisterra of the Medical Faculty of the Universidad del Valle and Dr. Rene Cruz of UNICEF have all been extremely helpful in setting up clinical trials and demonstration programs to establish the value of Incaparina under conditions prevailing in Colombia. This program was begun two years ago and has been invaluable in engendering enthusiasm among nutritionists and doctors for the product. Initial tests have continued on a demonstration basis in neighborhood clinics so that mothers can actually see the difference proper feeding makes in the health of their children.

Such demonstrations plus the enthusiastic endorsement of the product by these health authorities cannot fail to have an impact on the public image of the product. As soon as our production is adequate we hope to extend our program to institutional users which will further serve to introduce it to the consumer and provide implicit endorsement of its value.

In addition to these demonstrations we are also using an intensive advertising schedule, as shown in Figure 6. Radio is largely used as the medium since it is the most effective one for the Cali market.



El Público conocerá INCAPARINA, a través de la más completa Campaña Publicitaria !

- RADIO:

La Voz de Cali	360 cuñas cada mes
Radio Reloj	360 " " "
Radio Tricolor	360 " " "
La Voz del Valle	360 " " "

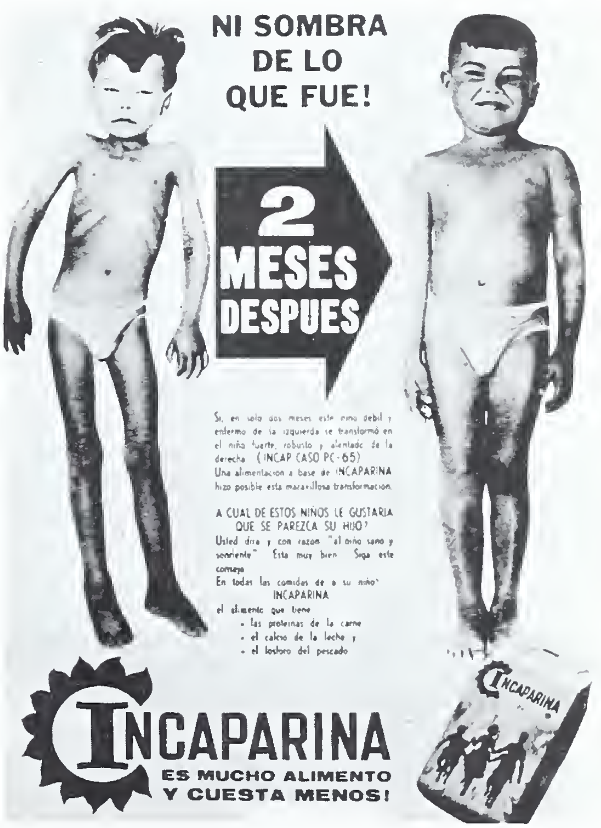
- VOLANTES
- AFICHES
- CINE y
- DEMOSTRACIONES AL PUBLICO EN
- Todos los barrios
- Hospitales
- Los Centros de Salud
- Escuelas
- Casa Comunal
- Centro Parroquial
- Fabricas y

CON EL CONCURSO DEL NIÑO SANO !

Figure 6

In all of the copy, nutritive benefits of the product are emphasized so as to bring about the education that is required. A film has also been prepared to show the problem of kwashiorkor and the value of Incaparina in its prevention. This film is used in a lecture, demonstration program by a trained nutritionist in the barrios as a further educational and promotional program.

The educational theme is also carried through the sales program. Information about the problem and its causes is a part of the sales brochure used in selling shopkeepers and the poster shown in Figure 7 is used as point of sale material, again pointing up the problem. It is of interest that while on a tour of the market place in Cali in November, one shopkeeper volunteered to us that he had discontinued selling platano flour and was selling Incaparina to those who asked for platano, instead.



NI SOMBRA DE LO QUE FUE!

2 MESES DESPUES

Si, en solo dos meses este niño debil y enfermo de la izquierda se transformó en el niño fuerte, robusto y alentado de la derecha (INCAP CASO PC-65)
Una alimentacion a base de INCAPARINA hizo posible esta maravillosa transformacion.

A CUAL DE ESTOS NIÑOS LE GUSTARIA QUE SE PAREZCA SU HIJO?
Usted dira y con razon "al niño sano y sonriente" Esta muy bien Siga este consejo
En todas las comidas de a su niño
INCAPARINA
el alimento que tiene
- las proteinas de la carne
- el calcio de la leche y
- el fosforo del pescado

INCAPARINA
ES MUCHO ALIMENTO Y CUESTA MENOS!

Figure 7

We have now had Incaparina on test market in Cali for about three months and initial results indicate that we have a successful product. If results continue favorable, we will expand the market nationally later this year.

While it is still too early to be sure, we feel confident that a protein-rich product partly based on cottonseed flour can be sold in the Colombian market. If it really is purchased in volume by the groups which need it most, and indications are that it is, then we feel sure that the product will have a significant impact on the public health problem of protein malnutrition. We believe that the concept and similar products can be successfully applied in other countries, as well.

Cottonseed flour is a logical choice for the protein supplement for many areas since the production of cotton is frequently coextensive with the nutritional problem. Furthermore, it is usually relatively cheap in price in comparison to usual foods because it has had, until recently, no use as a food. However, technical problems with the production of a satisfactory cottonseed flour exist which may retard such developments.

First, cottonseed meal in many underdeveloped areas is strictly a byproduct disposal problem. Many extraction plants are not properly equipped to reduce gossypol to low levels, still maintaining high protein quality. Repeated problems of excessive gossypol levels in Colombia led us to reduce the amount of cottonseed flour in Incaparina in favor of soy flour with which we could be assured of a more consistent product.

Second, the color of cottonseed flour limits its usefulness. Although the color is largely masked by panela in preparing colada from Incaparina, in many food products this is not possible. An important part of our effort to improve the diets of people in developing countries must be the supplementation of cereal flours used in preparing conventional dishes (6). The present color of cottonseed flour does not favor it for such use.

If a gland-free cottonseed, from which a white or light colored flour can be produced, can be developed for use in countries such as Colombia, it should greatly facilitate the expansion of cottonseed flour for food use. We hope that the further development of glandless cottons will be pushed with great vigor and that consideration will be given to exploitation of the development in countries outside the U. S.

In conclusion, it appears that the research efforts of the past few years have pointed the way towards a solution of one of the world's worst health problems--one affecting perhaps one-third of the world's children. We believe the time has come for commercial firms with production and marketing expertise to come to grips with this problem. We think that our initial experience in Colombia indicates that a product designed to provide protein to children can be effectively merchandised to people in a very low income bracket. Although conditions may be particularly favorable for a marketing success there, surely with government agencies and private enterprise working in concert, difficulties elsewhere will be surmounted. Certainly the magnitude of the problem and the risk of failure, are such that we must bring all possible resources to bear upon it.

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DISCUSSION

N. S. Scrimshaw: Thank you very much, Dr. Bruins. Your experience with the typographical error in panela reminds me of one of mine. When we were first developing the Incaparina formula, I had an opportunity to present the story and the results of the preliminary trials to an international conference held in Guatemala on the social and medical problems of the area. I confidently gave the formula which contained cottonseed flour, sesame flour, corn, Vitamin A and lime; the source of Vitamin A at that time was leaf meal, and the leaf meal which we were using was from Kikuyu grass. And so in Spanish, I listed "3 percent harina de kikuyu". At this, ripples spread through the audience and I thought they were certainly impressed with this contribution of INCAP. A bit later, I again mentioned the purpose of this 3 percent of kikuyu, and again a ripple spread through the audience. It wasn't until the meeting was over that I found that by pronouncing it "kykuyu" instead of "kikuyu," I was indicating that it was flour of ground-up insect grubs.

I am sure that the problems of the actual commercial introduction of a mixture of this sort after is has completed all of the laboratory, clinical and field trials, are of interest to this group. I will ask Mr. Shaw, who is the Economic Consultant to INCAP and who has been concerned with the commercial application in the various countries from the beginning to comment briefly on Dr. Bruin's account of experiences in Colombia, the preliminary experiences in Mexico and the general status of the commercial acceptability of Incaparina.

R. L. Shaw: You might have at least warned me about this. First, on sales; since the program started, we have a few figures on that. Sales went up 21 percent in '62 over '61 which was the first year that it was really attempted on even a small scale commercial basis. The '63 figures show a 93 percent increase over '62. That is accounted for by about 21 percent increase in sales in Guatemala during '63. In Mexico there is a beginning of the program there which is purely an acceptability trial program. This is a feeding program in one area of Mexico and involves 10,000 people.

What accounted for the balance of the increase in '63 was the beginning of the program that Dr. Bruins described which started late in September, ran three months during '63, and produced an average monthly sale which exceeded that of the monthly sales in Guatemala in spite of their increase. The Mexican program, and I comment on this very briefly, was an acceptability trial conducted by a Mexican producer in order to determine if this product was acceptable in rural Mexico. It was done in the area of Torreon in northern Mexico, and as I said, involved about 10,000 workers on a large construction project. Each day it was served to them as a regular meal and they were allowed to go back for any number of helpings they wanted. We found it was acceptable. It was also utilized and is being utilized in restaurants which a government corporation operates in that area for low income people. It was found acceptable there. On the basis of this, the Mexican producer is now planning his production with the view of distributing it through these same channels in the rest of Mexico. He is now working on the problem of getting the type of cottonseed flour he wants in Mexico.

In other countries it is still in the acceptability marketing period. We have authorized the distribution in Venezuela, Honduras, and Panama, and these are all in the acceptability stage.

N. S. Scrimshaw: Thank you very much. As a general comment, I might say that nutrition scientists, public health personnel, and personnel in international agencies concerned with nutrition, get very impatient with the lag between the development of a product of this sort and its establishment in practical use. It shouldn't be necessary to emphasize to this group that these things don't happen overnight, that it takes time and a great deal of work. I feel that some very promising formulas, mixtures, and low cost foods have been abandoned much too soon because the people responsible for developing them haven't had the know-how and the necessary commercial understanding to put them into practice. In the case of Incaparina we have been very fortunate in having persons adopt the concept, as the Quaker Oats Company has done in Colombia, and carry it forward into practical application. I might also say that although we have heard of many premature burials of the project and the product in the last few years, both the concept and the product seem to be, very much alive.

Just as we had one of the nutrition pioneers in Central America speaking to us earlier, we are very fortunate in having one of the outstanding food and nutrition scientists in South America to speak to us on this last topic. Dr. Antonio Bacigalupo has been responsible for some very significant advances in both basic and applied agricultural research in Peru. He has stimulated his associates; he has trained and guided students who have pioneered in research on the use of local food resources in animal feeding and in formulation of rations for this purpose, and in recent years has been concerned also with their use in human feeding. He has a deep understanding of the nature of the problem and the way it must be approached utilizing resources within Peru itself.

We are very pleased to have you with us, Dr. Bacigalupo.

EXPERIMENTAL PRODUCTION AND TESTING OF COTTONSEED FLOUR IN PERU[1]

by
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Lima, Peru
(Presented by Antonio Bacigalupo)

The Effect of Different Heat Treatments on the Biological Value of Cottonseed Flours

Introduction

A large proportion of the Peruvian population suffers from malnutrition due to low caloric and protein intakes. The Universidad Agraria in cooperation with the British American Hospital of Lima, has taken up a research and development program aimed at testing the feasibility of using cottonseed flour as one of several possible solutions to the national malnutrition problem. Bressani, Aguirre, and Scrimshaw (1) were successful in compounding a mixture of cottonseed and other vegetable flours to be used as a milk substitute in the feeding of children. Bradfield, Shankar, Fiala, and Barnes (2) and Bradfield, et al, (3, 4) recently published an evaluation of a Peruvian food supplement made from mixture of cottonseed flour and other flours prepared from locally available vegetable products. Workers at the Universidad Agraria, have developed formulas for poultry (5, 6, 7), and milk substitutes for new born calves (8, 26) based on a reprocessed cottonseed meal. Graham and Morales (9) have studied the problems of kwashiorkor and marasmus in young Peruvian children. These workers have used cottonseed flour in formulas for the treatment of these diseases with good results.

Ample evidence is therefore available to indicate the advisability of initiating production of cottonseed flour for human consumption. The production of cottonseed in Peru during 1960 was reported to be 220,000 metric tons (10), which would yield somewhat over 100,000 metric tons of cottonseed meal. Most of this meal is of poor quality due to the presence of a considerable proportion of hull and gossypol.

The first step in our work was to investigate the cottonseed processing industry as it presently exists in order to become familiar with the operations and the products over a wide geographical area. We found that among the different species and varieties of cotton grown in Peru, the "Del Cerro" variety, which is essentially the same as "Upland," produces a seed of relatively low gossypol content. This finding was important in allowing us to meet the WHO/FAO/UNICEF recommended maximum of one percent total gossypol without resorting to special solvent extraction methods. The free gossypol content of the product was lowered to within the limits recommended by the United Nations agencies by the use of a variation of the well-known moist heat method (11-23). As a source of cottonseed meal we chose a modern prepress solvent plant located within the "Del Cerro" producing area. This cottonseed meal was used in the experiments reported in this paper.

Materials and Methods

The prepress solvent plant operation which produced the cottonseed meal used as a starting material for our experiments possessed the following characteristics:

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[1] This work was supported in part by funds made available by the Institute of Arthritis and Metabolic Diseases, National Institute of Health. Department of Health, Education and Welfare Government of the United States of America, Gran Number AM-05935.

1. The hull separators were adjusted to remove a maximum amount of hull, thereby increasing the protein content of the meal. Both whole kernels and fines were included.

2. The meats were not preconditioned by addition of water.

3. The flakes were in the cookers for 18 minutes at 85° C.

4. The expellers worked at fairly low pressure thereby producing a cake containing about 18 percent oil.

5. After solvent (hexane) extraction and drying, the meal was found to have the following characteristics: 6. 6 percent moisture, 1. 2 percent oil, 49. 4 percent protein, 77 percent protein solubility, 0. 20 percent free gossypol, and 1. 1 percent total gossypol.

Proflo, a cottenseed flour recommended by representatives of the United Nations agencies and taken as a model in our work was obtained from the Traders Oil Mill Company, Fort Worth, Texas. It has the following characteristics: 10. 4 percent moisture, 4. 8 percent oil, 53. 1 percent protein, 59 percent protein solubility, 2. 70 percent available lysine, 0. 051 percent free gossypol, and 1. 0 percent total gossypol.

Free and Total Gossypol were determined using the official and tentative methods of the American Oil Chemists' Society. In order to normalize the gossypol data and eliminate the effects of varying moisture, oil, and fiber contents: free and total gossypol values were also expressed on the basis of protein as determined by the semi-micro Kjeldahl method.

Protein Solubility in 0. 02 N sodium hydroxide was determined by the method used by Lyman, et. al., (24).

Protein Efficiency Ratios (PER) were determined by dividing the average weight gain of a group of 10 male weanling Holtzman rats by the average protein consumption over a period of four weeks. The data were tested for statistical significance (27).

Available Lysine was determined by means of a slight modification of Carpenter's method (25).

Experimental

A cottonseed meal (A-1) was prepared at the plant in December 1962 from "Del Cerro" seed. The meats contained a minimum amount of hull and were processed in the usual way (see under methods and materials), with the exception that an additional amount of water was added at the time of cooking. The product had a yellow color, was relatively high in free gossypol, and at the 30 percent level in the diet of chick produced a mortality of 40 percent which on autopsy showed the typical gossypol toxicity picture in heart, liver, and kidney. For this reason A-1 was reprocessed by autoclaving during half an hour at 12 pounds per square inch. The vapors were produced by an electric water boiler which allowed for a slow rise in pressure and temperature, arriving at the required pressure at the end of 30 minutes. The brown, autoclaved product was designated A-2. A similar method of reprocessing was recommended by Lyman several years ago. It has been our experience that treatment with dry steam such as that generated in the boilers of the factory does not effect the desired change in the autoclaved meal. After autoclaving for more than an hour with dry steam, the meal within an inch or two of the surface undergoes browning, but the deeper regions remain unchanged. This observation suggested that the presence of water plays an important role in the process.

A second batch, B-1 identical with A-1, except with no water added during the cooking stage, was produced in June of 1963. Again, because of the high free gossypol, this product was reprocessed. The two ways used to accomplish this follow:

1. B-2 was prepared by autoclaving as described above for A-2, the difference being that the water content was adjusted to 25 percent before autoclaving. The color of the product was brown.

2. B-3 was prepared by first adding water carefully and with constant agitation to raise the humidity of the meal to 25 percent, and then drying with constant stirring in steam jacketed open kettles. The color of the product was tan. Two separate batches were prepared and tested in two different experiments.

The protein and gossypol contents of all these preparations appear in Table I. Comparing A-1 with B-1, we see that these two products are very similar. The latter is a bit lower in total and free gossypol. This may be due to some differences in the history of the batches of seed of which we are not aware, or it may be due to the fact that water was added to the cooker in the case of A-1. The protein solubility of A-1 is notably lower than that of B-1.

A-2 and B-2 are also similar, the difference being that in the case of the latter the humidity before autoclaving was 25 percent as opposed to ten percent in the case of the former. The presence of additional water in B-2 is believed to be responsible for the lower protein solubility and the lower free gossypol in the resulting autoclaved material.

B-3, produced from B-1 in an open, steam jacketed kettle, had slightly lower total protein and gossypol contents than Proflo. On the other hand, the protein solubility was considerably higher.

Since our ultimate goal has been to produce a cottonseed flour to be used in mixtures as substitutes for milk and other expensive protein concentrates, we have considered that the most meaningful tests are those which give us a measure of the actual protein utilization by the growing animal. Therefore, we adopted the following regimen: Holtzman male weanling rats were fed rations and given water ad libitum over a period of 4 weeks. Record of the weekly weight gains were kept. The average weight gain for each group of rats over the 4 week period was then used to determine the protein efficiency ratios. Allowing for the dependence of protein efficiency ratios on food consumption, these values can be considered a reliable measure of the biological value of the protein fed in the diet.

The rations used in the tests reported here are presented in Tables II, III, and IV. "Supplement No. 3" refers to a vegetable flour mixture prepared by Bradfield, et. al., (2, 4) and used in experiments with human populations in Peru. The mixture contained 41 percent cottonseed flour (Proflo), 30 percent broad bean (vicia faba) flour, and 20 percent quinua (chenopodium quinua) flour, 5 percent alfalfa leaf flour, 3 percent torula yeast, and 1 percent calcium carbonate.

The average growth of rats in each group, their total average weight gain, their total average food consumption on a dry weight basis, their total protein consumption, and their protein efficiency ratios (PER) are given in Tables V, VI, and VII.

In terms of PER the casein control was in all cases superior to the various preparations tested.

B-3 was significantly superior to both "Supplement No. 3" and B-2 and the difference between Proflo and A-2 was not significant.

DISCUSSION

Reprocessing by moist autoclaving or by adding water and drying at an elevated temperature at atmospheric pressure resulted in reduction of the amount of total gossypol. This finding seems to be in agreement with those of King, Frampton, and Altschul (18). Furthermore, the treatments described here were adequate to reduce the amount of free gossypol to within the range recommended by the WHO/FAO/UNICEF

TABLE I
Protein and Gossypol Contents of Cottonseed Meals and Flours Tested for Rat Growth

Prep. Base	% Total Protein (Weight)	% Soluble Protein	Total Gossypol (Dry Wt.)	% Free Gossypol (Dry Wt.)	% Total Gossypol (Protein)	% Free Gossypol (Protein)
A-1	52.3	67	1.4	0.25	2.4	0.48
A-2	47.5	58	1.0	0.060	1.9	0.11
B-1	49.4	77	1.2	0.21	2.3	0.41
B-2	47.4	45	0.87	0.023	1.9	0.044
B-3	50.3	73	0.94	0.037	1.8	0.070
Proflo	53.1	58	1.1	0.056	1.9	0.096

TABLE II
Percent Composition of Rations Fed to Rats in Experiment No. 1

	Lot No. 1	Lot No. 2	Lot No. 3
Casein (LM)*	13.22	-----	-----
Proflo	-----	18.69	-----
A-2	-----	-----	21.05
Vegetable Fat	9.96	9.08	9.31
Mineral Salts**	4.00	4.00	4.00
Sodium dihydrogen Phosphate	1.00	1.00	1.00
Choline Chloride	0.30	0.30	0.30
Vitamins (in cerelose)***	2.00	2.00	2.00
Sucrose	69.52	64.02	62.33
	100.00	100.00	100.00
Protein in Ration (Kjeldahl)	10.35	10.65	10.52
Fat in Ration (Soxhlet-hexane)	9.92	8.93	8.52

*Prepared in La Molina from fresh skimmed milk according to the method of M. S. Dunn in "Biochemical Preparations," Vol. I, ed. H. E. Carter.

**Mineral Salts	
$AlK(SO_4)_2 . 12H_2O$	0.017%
Calcium Carbonate	54.30
Copper Sulfate	0.090
Ferric Phosphate	2.050
Magnesium Carbonate	2.500
Magnesium Sulfate	1.600
Potassium Chloride	11.20
Potassium Iodide	0.011
KH_2PO_4	21.20
Manganese Sulfate	0.039
Sodium Chloride	6.900
Sodium Fluoride	0.100

***Vitamins in 2.0 g Cerelose	
Thiamine·HCl	0.40 mg
Riboflavine	0.80
Pyridoxine	0.40
Calcium Pantothenate	4.00
Niacin	4.00
Inositol	20.00
Folic Acid	0.20
Menadione Bisulfate	1.00
Biotin	0.02
Vitamin A	9.20
Vitamin B_{12}	0.003
dl-alpha-Tocopherol acetate 33%	15.00

TABLE III - Percent Composition of Rations Fed to Rats in Experiment No. 2

	Lot No. 1	Lot No. 2	Lot No. 3
Casein (LM)*	13.22	-----	-----
B-2	-----	21.08	-----
B-3	-----	-----	19.88
Vegetable Fat	9.96	9.70	9.75
Mineral Salts**	4.00	4.00	4.00
Sodium Dihydrogen Phosphate	1.00	1.00	1.00
Choline Chloride	0.30	0.30	0.30
Vitamins (in cerelose)***	2.00	2.00	2.00
Sucrose	69.52	61.92	63.07
	100.00	100.00	100.00
Protein in Ration (Kjeldahl)	10.69	10.24	10.55
Fat in Ration (Soxhlet-hexane)	10.36	9.98	9.13

* ** *** See Table II

TABLE IV - Percent Composition of Rations Fed to Rats in Experiment No. 3

	Lot No. 1	Lot No. 2	Lot No. 3
Casein (LM)*	13.22	-----	-----
B-3	-----	19.88	-----
"Supplement No. 3"	-----	-----	28.15
Vegetable Fat	9.96	9.75	8.49
Mineral Salts**	4.00	4.00	4.00
Sodium Dihydrogen Phosphate	1.00	1.00	1.00
Choline Chloride	0.30	0.30	0.30
Vitamins (in cerelose)***	2.00	2.00	2.00
Sucrose	69.52	63.07	56.06
	100.00	100.00	100.00
Protein in Ration (Kjeldahl)	10.39	9.98	10.17
Fat in Ration (Soxhlet-hexane)	9.50	10.32	9.48

* ** *** See Table II

TABLE V - Rat Feeding Experiment No. 1

	Lot No. 1	Lot No. 2	Lot No. 3
	(Casein)	(Proflo)	(A-2)
Weeks			
Initial	53.6 g	56.3 g	58.3
1	73.0	68.6	66.1
2	95.2	85.4	78.3
3	117.	101.	92.3
4	150.	116.	106.
Weight Gain	96.	60.	48.
Food Consumption	325.	345.	305.
Protein Consumption	33.6	37.8	32.1
PER	2.9	1.6	1.5

TABLE VI - Rat Feeding Experiment No. 2

	Lot No. 1	Lot No. 2	Lot No. 3
	(Casein)	(B-2)	(B-3 Batch 1)
Weeks			
Initial	47.5 g	50.9 g	51.7 g
1	66.0	66.3	68.7
2	90.0	78.7	93.2
3	112.	99.4	116.
4	147.	119.	136.
Weight Gain	100.	68.	84.
Food Consumption	324.	350.	367.
Protein Consumption	34.7	35.9	38.8
PER	2.9	1.9	2.2

TABLE VII - Rat Feeding Experiment No. 3

	Lot No. 1	Lot No. 2	Lot No. 3
	(Casein)	(B-3 Batch 2)	(Suppl. No. 3)
Weeks			
Initial	42.4 g	43.5 g	36.9 g
1	63.0	59.7	50.5
2	89.0	80.0	64.7
3	108.	103.	76.7
4	135.	124.	91.7
Weight Gain	93.	81.	54.8
Food Consumption	332.	357.	275.
Protein Consumption	34.5	35.6	28.0
PER	2.7	2.3	2.0

TABLE VIII - Comparison of B-3 with Proflo

	Proflo	B-3
Oil	5.36	1.38
Protein	53.1	50.3
Protein Solubility	58.	73.
Available Lysine	2.70	2.31
Total Gossypol on dry basis	1.1	0.95
Total Gossypol on protein basis	1.9	1.8
Free Gossypol on dry basis	0.057	0.037
Free Gossypol on protein basis	0.096	0.070
PER	1.6	2.3

for human consumption when "Del Cerro" cottonseed was used as the raw material.

One other factor which has not been mentioned before and which may play some role in determining the course of reaction of certain components of the cottonseed meal, is the presence or absence of air in the reaction vessel. All of the experiments in which the autoclave was used were essentially anaerobic, whereas in the experiments in which open steam jacketed kettles were used, the cottonseed meal was in contact with the air. We are planning further experiments to try to determine whether the presence or absence of air is important.

The results reported above definitely show that the method of applying heat and moisture does influence significantly the biological value of the cottonseed protein. It is interesting to note that the highly significant superiority of B-2 over A-2 can be accounted for only by the fact that during autoclaving the former was prepared with 2.5 times as much initial moisture as A-2. The protein solubility of B-2, however, is lower than that of A-2, so it is clear that in this case, the protein solubility data by itself does not predict the relative biological value of the two preparations.

Again, in the matter of the relation between available lysine and biological value, we have not seen evidence in our data to take a positive stand. B-3 was found to have an available lysine value of 2.31 as against 2.70 of Proflo.

Finally, our comparison of B-3 with "Supplement No. 3" strongly suggests that a protein supplement based entirely on a cottonseed flour such as B-3 would be more economical. Furthermore, it would be free from instability due to price fluctuations of the minor vegetable flour constituents.

Summary

This report deals with a preparation of a reprocessed cottonseed meal of high quality which shows considerable promise as a protein flour supplement for human use. A summary of the characteristics of this flour are compared with those of Proflo in Table VIII. Except where indicated otherwise, values were calculated on a dry weight basis.

Acknowledgments

The authors express their gratitude for the cheerful and dedicated technical service of Mr. Guillermo Chamorro and Miss Luisa Villacorta.

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DISCUSSION

N. S. Scrimshaw: I think it is interesting and fortunate that we have been given examples of two slightly different, but closely related approaches to the problem of obtaining a cottonseed meal or flour suitable for human consumption under the conditions prevailing in Latin America and other similar areas, Dr. Bressani concentrating on the initial processing in such a way that the product can be used, and now Dr. Bacigalupo on the preprocessing in a way that the product can be used for human consumption. The floor is open for discussion.

L. H. Bean: A minor detail in Dr. Bruins' presentation. I was wondering whether in your comparison, Dr. Bruins, of the costs per calorie in the United States and in Colombia the two sets of data are comparable. In other words, how did you make your comparisons for the United States? You said you used the Bureau of Labor Statistics prices for, say, all wheat not certain classes of wheat in the United States. Are the two sets of data strictly comparable?

H. W. Bruins: Let me say first to the data for Costa Rica that it was taken from some work done by Emma Reh and Claudia Fernandez, a dietary survey that was made in Costa Rica in 1950, and has been published by the INCAP group. In the U. S. figures we simply took the BLS figures of the retail prices of say chuck roast, pork chop, sausage, etc., and pooled them all on the same weighting value that is used in constructing the total index. In other words, we simply broke down each class in this fashion and assigned the caloric values to each one and then weighted them accordingly. Is that clear?

L. H. Bean: It is clear, but I am still wondering.

H. W. Bruins: I can't say that the two are completely comparable because we don't have really good consumption figures, particularly for the Latin American countries. Those are dietary surveys rather than economic statistics such as we use here in preparation of our figures. I am sure that the figures for the United States would be relatively more accurate than those for Costa Rica although they are both the basis of retail prices and both on the basis of total consumption for a particular type of product.

R. A. Hill: I would like to ask Dr. Bruins if he could give us any idea of the incongruousness that has been apparent in the first purchasing groups of the Incaparina in Colombia.

H. W. Bruins: So far we have only general impressions on this, however, the product does seem to be moving best in the markets serving the poorer barrios in Cali. The best class markets don't seem to have quite the movement that the lower class have. That is the best we can give you right at the moment. Follow-up surveys will be made and we will have a better idea of that in the future months.

N. S. Scrimshaw: I have been asked the question a number of times, why do we bother with cottonseed flour in these low cost mixtures when there is so much trouble with its processing and doubts as to the acceptable gossypol content. When it came to select the principal protein source for Incaparina in Central America, it would have been much easier, of course, to have taken soy flour for which the technology and human safety were much better established at the time. Soy was not, however, produced in quantity in the area. Although the agronomists were confident that it could be produced under these conditions, it was not, and is not today. Why not go to sesame? Again, there are problems connected with the harvesting of sesame, because the seed shatters; and the lower market price of the oil increased the price of the meal. For these reasons, our initial choice of sesame proved to be a poor one. Similarly, we eliminated all of the other potential sources of low cost protein, including fish protein concentrate.

Cottonseed flour seemed to be the best choice for a product which might come closest to competing with the price of the staple cereal, which in most of Central America was corn. We have seen that in Colombia it comes near to competing with the price of the basic staple there, which is rice. This also happens to be true for Panama.

From a nutritional point of view, of course, several of the oilseed meals are largely interchangeable. But from a standpoint of large scale production in areas in which this source of protein is most needed for human consumption, cottonseed flour does hold real promise and is worth all of the effort that is being put into it. It certainly is one of the most promising sources under conditions of real economic as well as nutritional need.

A. M. Altschul: Among the participants at this meeting is a distinguished visitor from India. We would like to take advantage of his presence and ask him to say a few words to this group. I have asked Dr. Max Milner if he will be good enough to introduce him. Dr. Milner.

Max Milner: Those of you who have had the opportunity to attend previous meetings of this kind, I think will be struck by the contrast of this meeting with some of the previous ones, as regards the number of visitors from abroad and the fact that there are more papers on the program dealing with oilseed protein studies in countries other than the United States. It is probably true that most of the scientific and technical efforts on cottonseed protein and its food uses have centered in the southern United States at institutions such as the Southern Utilization Research and Development Division, with the Texas group, and others. We in international agencies have been hoping for some time that other capable scientific groups will also become involved in this extremely important work. Much of our own effort has been directed toward assisting scientists and institutions in countries where these products will be utilized, in order to raise the level of their scientific and technological efforts so that they too can contribute to the total knowledge in the field.

This is the context then in which Dr. Sidhu is present at this meeting. He is the Director of the Regional Research Laboratory of Hyderabad, India. This is one of 26 largely applied science laboratories of the Council of Scientific and Industrial Research of the Government of India. I had the good fortune to visit this laboratory in Hyderabad a few years ago and I am sure that most of you who are used to a high level of research and engineering would feel right at home with what I saw there. It is a very comprehensive group covering engineering, chemical engineering, organic and biochemistry which has as its main objective getting processes into the market place for the benefit of the people of India. In a cooperative project with Dr. Sidhu, UNICEF has sponsored several of his senior people as fellows here at the Southern Utilization Research and Development Division, primarily in cottonseed technology. Dr. Sidhu is an organic chemist with undergraduate training in India and graduate training in Europe. I call on Dr. Sidhu for appropriate remarks. Will you come up, Dr. Sidhu? It is a pleasure to have you here, Sir.

REMARKS
by
G. S. Sidhu
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Thank you, Dr. Milner, for the kind words of introduction and thank you, Dr. Altschul, for giving me this opportunity of speaking about our work to this distinguished audience. I will not go into the need of protein supplements for India. The need is widely understood. The question is, what is the best source of proteins to supplement our diets? Here also the answer does not lie in one direction because of the magnitude of the problem. We have to tap all possible resources, and the oilseed proteins are one of the major resources to which we have now started paying a lot of attention.

Peanuts are the major oilseed crop in India, and the present annual production is at the level of about four million tons. The oil is consumed both in the raw as well as the hydrogenated state. Next in volume of seed production comes cottonseed of which we annually produce about 1.7 million tons; maybe this year it will be a little more, but unlike in U.S.A. it has barely been touched for the production of edible oil. There is little or no production of soya oil. There are other minor seed oils like safflower, coconut, but these are available in much smaller quantities.

The first oilseed to attract attention in this context was the peanut. The major effort in this direction has come from the Central Food Technological Research Institute, Mysore, one of our sister institutions, who has developed food formulations based on judicious blends of legume and peanut proteins or blends of wheat and peanut flour with skim milk. Two industrial units aided by UNICEF have been set up in India and are likely to be in production at any time now at a capacity of about 20 tons per day. The pilot plant at the Mysore institute has produced, over the last few years, a little over 2,000 tons of peanut flour which has been used in large scale experimental feeding trials particularly in school lunch programs. Now measures have been introduced to enrich at least 5 percent of the wheat flour produced in India. The present annual production of wheat flour in India in organized mills is about three million tons and 5 percent of this is to be enriched by addition of 10 percent peanut flour.

In this context we are also aware of the new complications produced by aflatoxin. So far the approach in edible peanut flour has been hand-picking of the seeds at different stages, and this will continue. We have also taken effective measures to find out the sources of fungal infection with a view to initiate appropriate prophylactic measures.

The next major source of oilseeds in India is cottonseed. Unlike in the United States, where the vegetable oil industry had its beginning in cottonseed, the traditional oils in India have been, as I have remarked earlier, peanut, sesame, mustard, safflower and coconut. The question has been asked by colleagues in private conversation, why is cottonseed oil not produced in India? The reason primarily is technological, and our indigenous village crushing equipment (ghanies) of the pestle and mortar type cannot crush cottonseed. Cottonseed was therefore not crushed but fed to cattle. Mechanizing the equipment was necessary. Modern expellers coupled with delinting, dehulling, etc., have now been set up, and there are many units which are crushing and further processing the cottonseed oil. Now the margarine that is made in India must have a certain portion of cottonseed oil in it. Last year about 7 percent of the oil for margarine was provided by cottonseed oil, and more of it will be used for this purpose now.

We started work in our laboratory about 14 years ago on different aspects of cottonseed technology. Our work over the past decade has naturally been inclined towards obtaining data on chemical composition of Indian cottonseed, storage of the seed, studies of cleaning, delinting of the Indian varieties of the seed, expelling, refining and bleaching of the crude oil and utilization of the soapstock. On this we have done fairly comprehensive work and industry has taken up this work. About five years ago we had a symposium at our Institute on cottonseed and its byproducts. The proceedings have been published. The data is now out of date by five or six years, but to those of you who are interested this will give the picture of cottonseed in India in 1958. I will leave this copy of the proceedings on the table for display. You may have a look at it, and we will be very happy to supply copies to those of you who may be interested.

It is only very recently that we have begun conducting studies on making edible flour from cottonseed. We have the other necessary units of processing up to 20 tons seed a day, but we had no equipment for grinding and air classification. Here we have had extensive help and cooperation from UNICEF in different ways. They have given us equipment. They have arranged for some of my colleagues to be trained in the United States. We also appreciate the hospitality of the U.S. Department of Agriculture and the cottonseed industry in this region, which has and is giving to my colleagues opportunities to work in the Southern Division where we are getting much useful assistance. We now expect to go into production for about half a ton of edible cottonseed flour per day.

The utilization of the cottonseed flour is a cooperative effort between three laboratories. Our part at the Regional Research Laboratory, Hyderabad, is the production of the cottonseed flour to standards which have been laid down and which are widely accepted, but we are not, in my institute, equipped to convert it into a diet. That work will be taken up by the Central Food Technological Research Institute, Mysore, who has had wide experience in this field and who has done much extension work in various parts of the country. The Nutritional Research Laboratory, Hyderabad will take our cottonseed flour and carry out nutrition and toxicity test on different types of animals. We look forward to further help from UNICEF in obtaining acceptance of this food supplement in various parts of the country. We do not expect any serious problem on food preference if this cottonseed flour is blended to the extent of 20 or 30 percent with wheat flour, because one of the most used items of food is a form of tortillas made out of wheat. Here is a fascinating area where we have done little research yet to study the effect of such formulations on leavening properties and the flavor of the resulting food products. In discussion with my colleagues at Mysore I have understood they will be paying special attention to these aspects.

Just one more thing and that is at the moment our food laws do not permit the use of extracted products for edible purposes because of the danger, real or imaginary, of carcinogenic residues in the solvents. However, petroleum refineries are ready to supply solvents and guarantee them free of aromatic residues; therefore, we should also get acutal data on solvent extracted flour by controlled feeding tests. Again, the role of my institute would be confined to providing this flour, and the feeding trials will be carried out elsewhere. We have made rough calculations as to the cost of production of cottonseed flour and we are confident that this will not exceed the cost of wheat flour.

In summary, I would say that we are very conscious of our protein deficit, and we want to work on this problem with speed and with all the resources at our command. Again I thank you for the most cordial welcome I have received and for inviting me and giving me the opportunity to benefit from the work reported at this conference.

Max Milner: Thank you, Dr. Sidhu, for your remarks. I am sure that all of us now have a better appreciation of the implication of the things that we are talking about these few days, and the fact that they are being taken up and applied in a very useful way in many parts of the world. Mr. Chairman.

A. M. Altschul: I, too, want to thank you, Dr. Sidhu, for your remarks and for coming to this meeting. We are happy to know that you are enjoying the discussions and benefiting from them. And now, we are ready for the afternoon session.

L. A. Goldblatt: Yesterday we heard a good deal about the basic chemistry, the organization, the structure of cottonseed. That may provide the basis for a cottonseed processing technology in a future generation. This afternoon, we are down to the practical problem of producing cottonseed protein concentrates. Yesterday vou heard from Garlon Harper about the revolution that has taken place in cottonseed processing since World War II. In 1946, about 95 percent of all our cottonseed was processed by hydraulic processing. Now it is down to about 3 percent. That represents a change from 95 percent to about 3 percent in less than 20 years. And today, we are on the verge of a new revolution. I am intrigued by Dr. Beans' quotation yesterday, "My heart leaps up when I behold a rainbow in the sky." The fortunate scientist also sometimes sees a rainbow in his sky and traditionally every rainbow has a pot of gold at the end; the entrepreneur may be tempted to seek that. Probably all of us have seen at one time or another a double rainbow; and I recall on one occasion having seen a triple rainbow. We are now in that position with respect to cottonseed processing. We are in the fortunate position of having a choice of several new promising procedures, especially new solvent systems, and there is a promise of still more to come. The first of the new systems that is to be presented, is the one that was developed at the Southern Regional Research Laboratory in New Orleans. It will be presented by Dr. Frampton.

REVIEW OF THE MIXED SOLVENT PROCESS FOR EXTRACTING COTTONSEED

by
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Cottonseed flakes are cooked in the conventional processing operations that are followed in oil extraction. The meats are cooked irrespective of the process used in extracting the oil--whether it is the hydraulic press, the screw press, or extraction with petroleum naphtha--and the cooking operation is carried out to render the oil. Oil yields are unsatisfactory unless the flakes are cooked.

We know from our discussions yesterday that we must not cook cottonseed flakes if we are to produce cottonseed meals of the highest attainable nutritional quality. It was established, for example, that cooking destroys a portion of the lysine, and, moreover, a portion of the lysine that escapes destruction is bound in a form that is not available to broilers, rats, or swine. It is also known that gossypol is bound in the meal by the cooking process--some gossypol is bound to the epsilon amino group of lysine, some is bound in other forms.

We must not cook cottonseed flakes! The cooking process must be circumvented and we must extract cottonseed oil from raw flakes. This means that we must depart radically from conventional methods and techniques that are used in the vegetable oil industry.

Yesterday, Dr. Yatsu, Dr. Wall, and Mrs. Martinez discussed the anatomy of the cottonseed and cottonseed cell, as observed from prepared specimens. I want to extend that discussion briefly by referring to the functions of some of the structures that appeared in the micrographs. It must be understood at the outset that one of the most important constituents of cells was not depicted in the micrograph or considered in the discussions. This important constituent is water. Most of us take water for granted. It should be emphasized that protoplasm is a hydrophylic gel--a gel where water is the continuous phase and all of the other cell constituents are dispersed in a water matrix. All life processes take place in aqueous solution, and when I speak of the cell solution, I am referring to an aqueous solution; when I speak of a dispersion in the cell, I am referring to a system where water is the dispersion's medium.

There are many interfaces within the cell, as you noted in the micrographs that were presented. The membranes in these cells, and the structures that we saw, arise through the capillary activity of substances in the cell solution; these capillary active substances, substances like proteins, glycoproteins and phospholipids, etc., concentrate in the interfaces in accordance with the requirements of the Gibbs equation,

$$C_s = (dr/dC)(-C)/RT.$$

Here, C is the concentration of a constituent in the cell solution, r is the interfacial tension, T is the absolute temperature, R is the gas constant and C_s is the concentration of of the solute in the interface. The capillary substances not only concentrate in the interfaces, they are oriented in the interface in orderly arrays, and in a manner that is dictated by the fundamental laws of thermodynamics. These interfacial films are the organized membrane-like structures that are fundamental to the organization and functioning of the living cell. These structures in the living cell are not static. They are subject to continual modification and change, depending upon the physiological activity within the cell and its environment. As we watch a living plant cell under the microscope, we see very active protoplasmic streaming within the cell, as the contents of the cell are in continuous flow.

The structure of major importance to our problem of oil extraction is the outermost structure of the living cell. This is referred to commonly as the plasma membrane, and it is composed chiefly of proteins, glycol proteins and phospholipids, etc. This is a semipermeable membrane and it mediates the passage of all substances to and from the living cell. Changes in the composition of the plasma membrane very seriously modify the properties of the membrane and drastically modify the physiological activity within the cell. The cooking operations carried out in commercial cottonseed processing destroy this membrane.

A striking demonstration of the effects of changing the composition of the plasma membrane is found in the response of living cells to the anesthetics--substances like diethyl ether, acetone, and chloroform. These polar substances are soluble in the cell solution, and they are also capillary active and concentrate in the interfaces. When this happens the nature of the interfacial membrane is altered, and this is reflected in the altered activity within the cell. The exposure of an actively photosynthesizing leaf to diethyl ether, for example, causes the immediate release of copious quantities of enzymatically bound carbon dioxide. The evolution of CO_2 from the leaf is almost explosive. The injection of diethyl ether into an animal tissue, or plant tissue, causes instantaneous necrosis, and the necrosis is indistinguishable from that obtained when the tissue is badly burned or deeply frozen. Nonanesthetics, like the fats or vegetable oils or heavy paraffin oils, are innocuous. For example, chemotherapeutic substances like penicillin are administered intermuscularly in solution in sesame oil.

A further indication of the effectiveness of anesthetics in modifying the cell membranes and rendering oil is found in the fact that quantitative extractions of fat from animal tissues are effected only with polar solvents, e.g., solvents that are water soluble and are capillary active. One normally uses chloroform and alcohol, or diethyl ether, or acetone. He never uses petroleum naphtha, which is water insoluble and capillarly inactive.

Our problem in extracting cottonseed oil from raw flakes is fundamentally one of destroying the organization of the cells and the plasma membrane, and other membranes, without the use of heat. The first efforts we made at extraction, with the view of maintaining the native level of available lysine, were with a polar solvent--namely acetone. There is, however, a limited usefulness of acetone in that the solubility of cottonseed oil in acetone at ambient temperatures is strongly dependent upon the water content of the acetone. Cottonseed oil is virtually insoluble in 95 percent aqueous acetone. We added petroleum naphtha to the acetone to circumvent this difficulty, and then we saturated the mixture of these two liquids with water to retain or increase the affinity of the plasma membrane for the solvent mixture.

The action of this solvent mixture in rendering the oil is most spectacular. On making observations under the microscope, within five seconds after contact is made between solvent (57 parts acetone, 39 parts hexane, 4 parts water by weight) and seed fragment, one can see jets of oil shooting from the cells in the surface of the seed fragment. The hydrophylic components of the solvent mixture penetrate the plasma membrane by a process of imbibition, similar to that noted when a cell is placed in an hypotonic aqueous solution. Here we do not get a swelling of the cell and and its final rupture because of the inflow of water, but rather the pressure, caused by the influx of components of the solvent, is released by the flow of oil from the cells through the disorganized plasma membrane. All jet activity subsides in about 60 seconds.

The behavior of the pigment glands is of special interest. These bodies are also rapidly emptied of their contents, not through the rupture of the membranes surrounding the bodies, but in the same manner that the cells of the cotyledon are emptied of their oil. The yellow pigment glands disappear completely. Red and purple bodies remain in the fragment of seed after the oil has been extracted, but when they are teased from the tissue with a dissecting needle, they are found to be colored ghosts of red and purple pigment glands--only hulls remain that are completely empty. These hulls are composed in part of protein and they contain some bound gossypol.

A comparison of the rate of oil and gossypol extraction by hexane, acetone, and the solvent mixture is found in Figure 1.

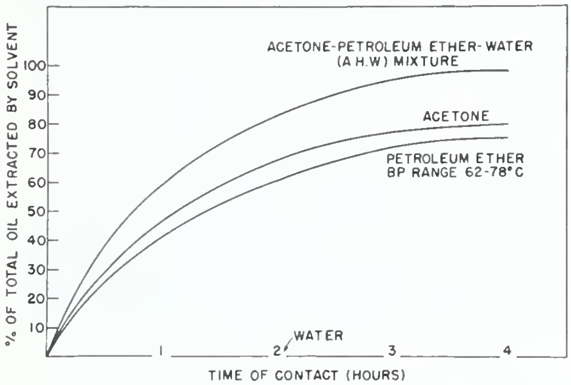

Figure 1. A comparison of the rate of oil extraction by hexane, acetone and the solvent mixture.

The data were obtained by contacting three parts of the solvent and one part by weight of raw cottonseed flakes and analyzing aliquots of the supernatant liquid at intervals. Note that under these conditions the percent of the total oil in the flakes that is extracted by the solvent mixture approaches 100 percent as a limit--that extracted with hexane only approaches about 75 percent of the total oil. The percentage of extractable gossypol (Figure 2) extracted by the mixed solvent approached 100 percent, while the limit approach with acetone is only about 60 percent. Hexane extracts no gossypol.

Our basic information shows that good extraction may be obtained with acetone and hexane mixtures of various proportions, providing the mixture is saturated with water. The effectiveness of the solvent decreases as the degree of saturation with water decreases. The effect of solvent composition on the solvent properties is illustrated by the data included in Figure 3, where the solvent power of a mixture composed of acetone, hexane, and water in the

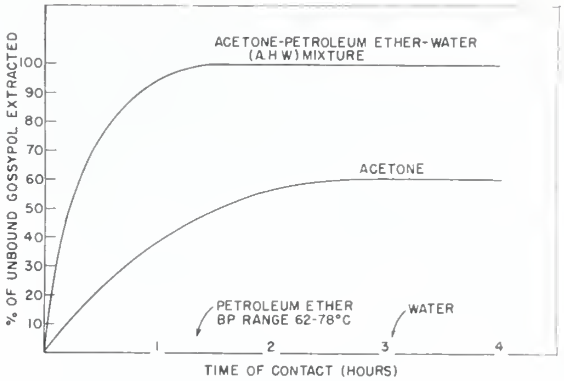

Figure 2. A comparison of the rate of gossypol extraction by hexane, acetone and the solvent mixture.

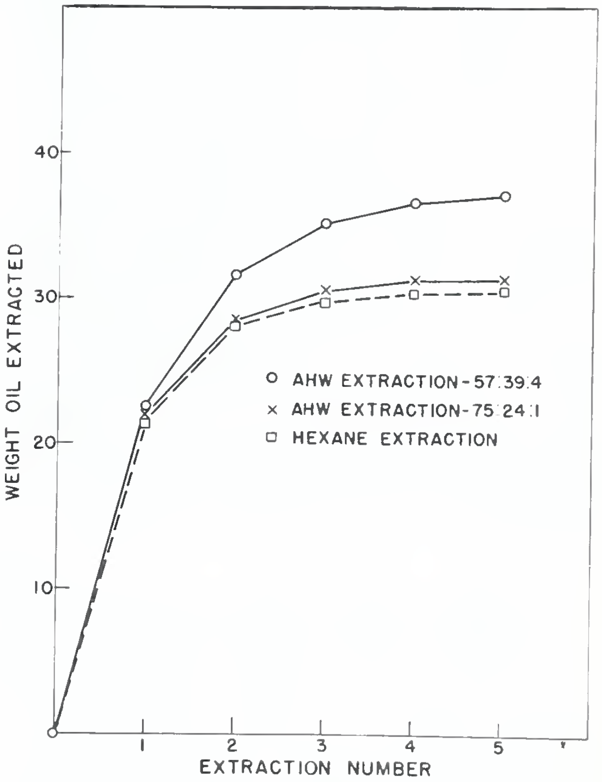

Figure 3. The effect of solvent composition on solvent properties.

ratio by weight of 57:39:4 is compared with a solvent mixture composed of acetone, hexane, and water in the ratio of 75:24:1. Data obtained with hexane alone are included for comparison. The flakes were suspended in three volumes of solvent and the suspension was permitted to stand for four minutes, with occasional stirring. The solvent was then decanted and the marc was permitted to drain for one minute. An aliquot of the miscella was taken for analysis, and the marc was suspended in three volumes of fresh solvent, and the process repeated. Five extractions were made. Note the very considerable difference between the solvent power of the two solvent mixtures. The one is not much better than hexane alone, while the other is markedly superior to hexane alone.

Results of experiments to compare the total amount of neutral oil obtained on exhaustive extraction with the solvent mixture composed of acetone, hexane, and water in the ratio of 57:39:4 by weight are shown in Table I. The crude oil content of the meats, determined by the official method of the American Oil Chemists' Society, was found to be 26.4 percent with this sample of meats. Exhaustive extraction with petroleum naphtha (b. 67-71°C.) yielded 24.7 percent neutral oil. Exhaustive extraction with the solvent mixture referred to above yielded 25.6 percent neutral oil. The difference noted here in the yield of neutral oil corresponds to about 13 pounds per ton of cottonseed. This is a bonus that can be realized when the mixed solvent is used for processing cottonseed.

Of immediate interest are the oil color and refinability of oils obtained with the mixed solvents. Three different procedures for refining the oil have been carried out on a pilot-plant scale. In the one, the solvent was removed from the oil by conventional distillation and stripping. Refining data obtained with aqueous sodium hydroxide (containing 17.1 percent by weight NaOH) are shown in Table II. Half a gram of sodium hydroxide in excess of that required to neutralize the fatty acids and gossypol was used per 100 grams of crude oil. Refined oil yield equal to 97.6 percent of the total neutral oil present was obtained. The oil was bleached following the official procedures of the American Oil Chemists' Society to yield a very light colored oil with a color index of 17 on our color index scale.[1/] This color corresponds roughly to an index 1.7 on the official American Oil Chemists' Society photometric color scale.

A second procedure for refining the oil consisted in converting the acetone-hexane-water, oil miscella, to a hexane oil miscells by extracting the acetone and gums with water. This was done on a pilot-plant scale. Six portions of water, equal to 10 percent of the volume of the miscella, were used to extract the acetone and water extractable materials. Yields of refined oil and oil color are noted in Table III. Very good yields of refined oils, which bleach to oils of very light color, may be noted from the data reported in Table III.

There are no engineering difficulties in recovering acetone from the water phase, since it can be recovered by azeotrope distillation. Acetone, hexane, and water form an azeotrope which boils at 49°C., and consists of acetone, hexane, and water in the ratio of 35.5:35.5:1.5 by weight.

A third procedure for refining the oil is that of refining in the acetone, hexane, water, oil miscella. This will be discussed in the section dealing with the work being carried on by Mr. E. A. Gastrock.

Typical data for available lysine in the mixed solvent meals are shown in Table IV. Data obtained with typical commercial cottonseed meals are included for comparison.

Data for gossypol are reported in Table V. Note the level of total gossypol in these meals.

Meals containing high levels of available lysine and low levels of total gossypol were subjected to a series of feeding tests. Several lots of meal were sent to UNICEF

1/ Pons, W. A., Jr., Cook, J., and Frampton, V. L., Color Index for Cottonseed Oils, J. Am. Oil Chem. Soc., 27, pp. 671-675.

TABLE I

Extraction of Oil from Raw Cottonseed Flakes

Solvent	Oil Removed % of Meats					
	Crude			Neutral		
	1	2	3	1	2	3
Petr. ether b. 35-60°C.	26.4	26.5	-	-	-	-
Petr. ether b. 67-71°C.	27.1	26.4	27.1	25.4	24.8	24.0
Azeotrope	34.3	33.0	33.0	26.3	26.3	25.6

TABLE II

Refining of Crude Oil (AHW Solvent Distilled from Miscella)

Total Acidic Substances in Crude Oil	Excess NaOH per 100 g. Crude Oil	Yield of Oil		Color of Oil (1)	
		Refined	Neutral	Refined	Bleached
4.5	-	-	89.3	-	-
4.5	1.5	86.3	-	73	12
4.5	0.5	87.2	-	80	17
4.5	0.0	-	-	190	100

TABLE III

Refining of Crude Oil
(Water Washed Azeotrope Miscella)

Total Acidic Substances in Crude Oil	Excess NaOH per 100 g. of Crude Oil	Yield Refined Oil	Color (1)	
			Refined	Bleached
7.0	5.6	93	117	25
5.6	10.2	95	103	20
7.8	4.4	93	83	13
8.4	3.1	92	96	18
4.3	1.6	96	273	218

TABLE IV. Available Lysine in Azeotrope Meals

	Screwpress	Prepress	Hexane	AHW
	2.59	3.14	3.30	4.23
	3.07	3.21	3.31	4.18
	2.70	3.38	3.20	4.15
	3.04	3.70	2.82	4.18
	3.22	3.60	3.67	4.26
	3.43	3.17	3.64	4.25
	2.22	3.53	3.75	4.25
	3.11	3.36	3.12	4.28
Average:	2.84	3.35	3.39	4.22

TABLE V. Analyses of Finished Azeotrope Meals

Sample	Free Gossypol	Total Gossypol
M-1	0.03	0.12
M-2	0.03	0.11
M-3	0.04	0.14
M-4	0.03	0.11

over a period of three or four years. Dr. Milner made reference yesterday morning to the results of feeding tests with these meals. As a matter of fact, all of the data show that the meals produced by the mixed solvent process are markedly superior to conventional commercial cottonseed meals. There has never been an instance where tests have shown any but markedly superior results. Protein efficiency ratios were observed to range from 2.2 to 2.6. In no case have stored shell eggs produced by laying hens fed rations containing 20 percent of the mixed solvent meal prepared in my laboratory been found to be abnormal; in no case have we been able to find the gossypol induced chromogen in the yolks; in no case have we found pink whites. These meals, in limited tests, have killed no pigs.

In view of the results of these biological tests and the observation that oil yields are good and that there is no serious problem in oil refining and bleaching, attention was given to the practical problem of producing these meals on a larger scale.

The major problem, from a practical point of view, is that of extraction. The problem arises because of the plasticity of the marcs produced with solvent systems that are saturated with water.

Two approaches to the extraction phase of the method have been followed. The one under my supervision has as its objective the development of the most economical procedure for the extraction of oil from raw cottonseed flakes without regard to existing industry practices and the equipment presently used for extraction.

The second approach has been under Mr. E.A. Gastrock's leadership, where he has been interested in adopting the mixed solvent process to plants equipped with basket extractors. I want to discuss his work first. It is through Mr. Gastrock's courtesy that I am outlining his report. Mr. Henry Vix will comment on phases of the work at the conclusion of my paper.

Mr. Gastrock's solvent of choice is composed of acetone, hexane, and water in the ratio of 39:60:1 by weight, and the process is outlined in Figure 2 of the manuscript that was passed out to you. The usual delintering and decortication procedures are used, where emphasis is placed on as complete a separation of hulls and meats as possible to produce a meal of high protein content.

The flakes one has to deal with in a solvent system saturated with water are soft and they have a tendency to form a plastic mass. It is for this reason that the meats are tempered in conventional equipment under mild conditions. The meats are tempered at 10.5 - 11 percent water for 30 minutes and then flaked to a thickness of 0.006". The flakes are aspirated into a stream of warm air--140°F.--for about 30 seconds, separated in a cyclone separator and cooled to about 100°F. The resulting flakes are somewhat crisp rather than flexible and provide satisfactory percolation rates in the basket extractor.

As shown in the flowsheet, extraction is in two countercurrent units. The first is a conventional basket extractor of the horizontal, rectangular or (probably also) of the deeper bed stationary or rotary basket type. Three stages of extraction should suffice, and because commercial units contain 16 to 34 or more baskets, a suitable combination can be easily devised to achieve the three stages.

The second unit is a total immersion type extractor, providing 15-20 minutes of countercurrent contact in the horizontal screw section, followed by free suspension of the marc particles in the lower portion of the inclined drag part of the extractor. Before the inclined drag removes the marc from the extractor, the marc passes over a perforated screen section which permits free solvent to drain from the marc.

Half of the fresh solvent enters the second unit just above the level in the inclined section and the other half at the base of the drag after washing down the fines that pass through the drainage screen. Half miscella overflow at the end of the horizontal screw section and most of the fines are removed to the settling compartment and returned to the marc discharge end of the horizontal unit.

Settled half miscella is the extracting solvent in the basket unit, usually with three countercurrent stages as shown. Part of the product miscella is refiltered through the baskets as shown.

The extracted marc has been successfully desolventized in standard Schneckens type desolventizers without recourse to vacuum, using some direct steam in the early stages. Maximum meal temperature of 205°-210° F. does not have an adverse effect on the available lysine as determined by the DNFB (dinitrofluorobenzene) method.

The product miscella is passed through a polishing filter and then concentrated to about 50 percent oil content, and is refined batchwise. The soapstock is separated in a continuous, vapor-tight centrifuge. Clean separations are obtained without water washings. The refined miscella is filtered with a small amount of filter aid and is further concentrated and stripped to yield a prime bleachable refined oil. All solvent streams are returned to the make-up tank. Hexane and acetone to compensate for losses also enter this tank subject to control signals. Control instruments of the capacitance type monitor the upper layer--or saturated layer from the centrifuge--calling for additional hexane or acetone for correction purposes, as the case may be. The lower layer, containing the excess water picked up by the miscella during extraction and by the solvent during desolventizing, passed to the rectifying columns. Because the system is designed to operate on a mixture of hexane and acetone saturated with water, the overhead product from the rectifying columns may contain as much as 10 percent water.

The work tank must be large enough to hold all the mixed solvent when the plant is shut down or laid by. The operating level is low in the tank and at a certain minimum point controls function to admit hexane and acetone from the supply tank in the proper proportion until the operating level is reached.

Properties of meal, crude miscella and refined oil determined by Mr. Gastrock's group are reported in Table I of the next paper. Note the high level of available lysine, the low level of total gossypol, as well as the increased yield of bleachable oil over that obtained by conventional hexane extraction.

Cost estimates for converting existing solvent plants with horizontal basket extractors are shown in Tables II, III, and IV of the following paper.

As I mentioned previously, my group is interested in developing the most economical procedure for extracting cottonseed with solvent mixtures composed of acetone, hexane, and water. The results of our experiments led us to concentrate originally on a solvent mixture composed of 57:39:4 parts by weight of acetone, hexane, and water. However, variations from this composition make only small differences in the effectiveness of the solvent in removing oil and gossypol from raw moist flakes; the chief requirement is that the solvent mixture be saturated with water.

As noted previously, acetone, hexane, and water form an azeotrope that boils at 49° C. This azeotrope is composed of 35.5:35.5:1.5 parts by weight of acetone, hexane, and water. The azeotrope is also an excellent solvent for oil, and it is almost as good as the best solvent mixture used for extracting oil and gossypol from raw cottonseed flakes in pilot plant experiments. The azeotrope is our solvent of choice, chiefly because it is an azeotrope. It can be handled in the process as though it were composed of a single component. Many of the problems of solvent recovery, solvent remake, oil desolventizing and meal desolventizing that are naturally inherent in a process using a mixed solvent are very largely circumvented by the use of the azeotrope.

Our data show that the fastest and most complete extraction of oil and gossypol from raw cottonseed flakes is obtained when the fresh flakes entering the production stream are wet. They should contain at least 12 percent water. The fact that wet (water) cottonseed flakes which are moistened with a solvent mixture that is saturated with water, will form a plastic mass if they are subjected to pressure dictates a method for separation of miscella and marc where deep beds of flakes are avoided. A vibrating screen separator that can handle thin layers of flakes or marc in a continuous stream provides a means for the rapid extraction of oil from the plastic marc. The vibrating action of the screen keeps the clumps of flakes separate and thereby it facilitates the drainage of the miscella through the screen. It is immaterial how wet or plastic the marc is.

In the operation the moist flakes (12 - 15 percent water by weight) are discharged directly from the flaking rolls into the screw conveyor shown in Figure 4, simultaneously with a stream of the azeotrope. This conveyor and vibrating screen, shown in Figure 4, make up the basic unit in the extraction procedure I am talking about. The flakes and azeotrope are mixed as they both move down the screw conveyor. The rates of feed of the flakes into the screw conveyor are adjusted so that a layer of flakes no more than 1/2 inch thick is built up on the screen. The ratio of solvent to flakes for most of the experiments we have carried out is 3:1. The miscella and marc are discharged from the discharge port of the screw conveyor onto the center of a circular vibrating screen (160 mesh). This self-cleaning screen forms the bottom of a shallow cylinder--or rather it forms the partition between a shallow cylinder and a shallow pan with an outlet port. The vibrating unit is mounted on a stationary frame by a series of coil springs, and the vibrating action is produced by a small electric motor that is fastened to the bottom of the unit. The motor is thrown off balance by a series of weights that are attached to the shaft, and the gyrations are adjusted so that the marc moves radially to the periphery of the screen, and then along the cylinder wall to the discharge port of the upper chamber. The miscella drains through the screen to the lower chamber and is discharged through the port. A second screw conveyor receives the marc from the first screen, together with the next miscella, and these are mixed as they move down the screw conveyor to the next vibrating screen unit.

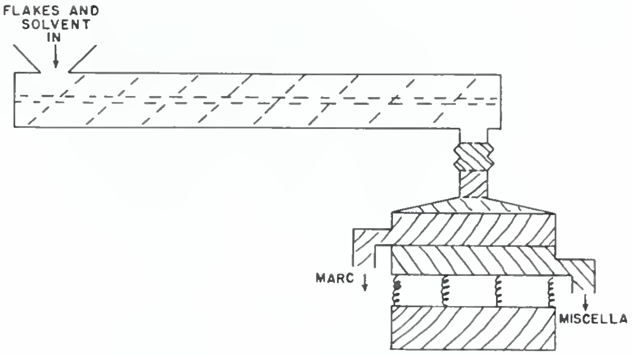

Figure 4. A vibrating screen assembly.

The vibrating screen apparatus, which is available commercially, will operate indefinitely without clogging, irrespective of the degree of plasticity of the marc or the rate at which it is fed.

The residence time of the marc and miscella in our basic unit (screw conveyor and screen) is 17 seconds.

The product miscella from the countercurrent extraction is discharged through the port of the chamber under the screen into a settling tank to permit the screenings to settle. These screenings may make up as much as 10 percent by weight of the original flakes, depending on the manner in which the flakes are prepared originally. However, they are dense because of the moisture they carry and they settle very rapidly. They are removed from the bottom of the settling tank and are recycled by returning them to the stream with the fresh flakes. Protracted runs have established that the screenings do not build up in the product miscella.

The observations of the behavior of a seed fragment suspended in the solvent mixture, as viewed under the microscope, suggest that the oil may be extracted from raw moist flakes in a matter of seconds. This prediciton was verified in experiments conducted on a pilot-plant scale. Analyses were carried out on the marcs and miscellas from each step in a 10-step countercurrent extraction. The composition of the marcs, as calculated from the data is shown in Table VI.

It is obvious from these data that the extraction of oil from the cottonseed cells is complete and quantitative in three steps of the countercurrent extraction. The subsequent steps in the countercurrent extraction with the mixed solvent consist in efforts to rinse oil from the marc with oil rich miscella.

The conclusion that the oil is extracted quantitatively in a very brief period has been confirmed repeatedly. The results from a 4-step countercurrent extraction are reported in Table V as an illustration of the kind of results that can be obtained. Ten pounds of flakes--taken directly from the rolls, and without any prior treatment--were moistened with 1. 5 volume of the fresh azeotrope, and then immediately fed into the screw conveyor along with a miscella No. 3 (oil rich) that had been used three times in a previous 4-step countercurrent extraction. The flakes and miscella were mixed as they moved down the conveyor and were discharged onto the screen, with the combined residence time in conveyor and screen of 17 seconds. The marc from the vibrating screen was pressed briefly between canvas cloths at 100 p. s. i., and samples of press cake, marc, and miscella were taken for analyses. The remainder of the marc was fed, together with miscella No. 2 (miscella used twice) into the screw conveyor and mixed in the manner previously noted. The marc discharged from the screen was again pressed briefly at 100 p. s. i. Samples of marc, press cake and miscella were taken for analyses. The marc was then extracted in the prescribed manner with miscella No. 1 (used only once), and appropriate samples were taken for analyses. Finally, the last marc was extracted in the prescribed manner with three volumes of fresh azeotrope. The final marc, miscella No. 1 and press cake were taken for analyses. The final press cake will contain about 17 percent water and about 3 percent of azeotrope. The compositions of the air-dried meals from each step and the time required for each step in the operation are given in Table VII. Note that the residual oil in the product meal, as determined by the Official Methods of the American Oil Chemists' Society, is less than 0. 1 percent. The total gossypol for this extraction was reduced to about 0. 24 percent.

Miscella No. 3, No. 2, and No. 1 are low in oil. Normally, miscella No. 3 (the richest miscella) will contain about 0. 6 percent oil; miscella No. 2 will contain about 0. 2 percent oil; and miscella No. 1 will contain less than 0. 1 percent oil. Ninety-nine percent of the oil extracted by this process is extracted in miscella No. 3.

Whereas ratios of volume of solvent to flakes in these extractions has been of the order of 3:1, it is evident from bench-scale experiments that this ratio can be materially reduced and still give virtually quantitative extractions of oil.

The extraction reported in Table VII for a 4-step countercurrent extraction that consumed a total of three minutes extraction time was carried out in a pilot-plant operation where the improvisations of small pilot-plant operations have to be tolerated. Our data from bench-scale experiments make it seem probable that comparable extraction

TABLE VI. Rate of Oil Extraction in a Countercurrent Operation-composition of Marc % Crude Oil

Step #	% Solvent	Total	In Adhering Solvent	Original Oil Remaining In Meal	% Original Oil In Air Dried Meal on Dry Basis
Flakes	-	-	-	-	30. 6
1	51	8	6	2	4.5
2	54	7	6	1	2.5
3	60	5	5	0. 0	0.0
4	61	4. 5	4. 5	0. 0	0.0
'	'	'	'	'	'
'	'	'	'	'	'
'	'	'	'	'	'
'	'	'	'	'	'
'	'	'	'	'	'
10	68	0. 8	0. 8	0. 0	0.0

TABLE VII. Product Obtained in a Four-Step Countercurrent Extraction with the Azeotrope

Step	Operation	Residence Time	% Total Gossypol	% Oil
0	Raw flakes	0	1. 1	30. 6
1	Contact with #3 miscella, ext. on screen light pressing 100 p. s. i.	60 seconds	0. 45	2. 8
2	Contact with #2 miscella, ext. on screen, light pressing	40 seconds	0. 34	1. 2
3	Contact with miscella #1 ext. on screen, light pressing	40 seconds	0. 28	0. 48
4	Contact with fresh solvent ext. on screen, press at 400 p. s. i.	40 seconds	0. 24	0. 1

Total Residence Time 180 Seconds

in the pilot plant can be achieved in a 3-step extraction in two minutes or less.

The capacity of the small screen (9-inch radius) in our basic unit was determined experimentally to be ten tons of cottonseed per day. The pace setting step is in the initial extraction of the flakes with miscella No. 3 because of the high viscosity of the product miscella that must be drained away. The capacity of a circular screen increases as the square of the radius, so a circular vibrating screen separator with a radius of 15 inches should process about 28 tons of cottonseed per day and one with a radius of 24 inches should process about 71 tons of cottonseed per day.

The conversion of existing prepress solvent and solvent plants to azeotrope plants with vibrating screen separators should be relatively simple. Existing flaking rolls, solvent recovery system and meal desolventizing installations should suffice, with only minor alterations. Existing facilities for refining the oil should be adequate.

The cost advantage in eliminating the cooking operation is not estimated in this discussion of the use of the vibrating screens in extracting raw moist cottonseed flakes with the azeotrope. The cost advantage that accrues because of the partial desolventization of the marc in a light pressing operation is also not estimated. The cost advantage gained through a greater yield of neutral oil is probably equivalent to 12-15 pounds more neutral oil per ton of seed over that obtainable with hexane. The cost difference in handling the azeotrope in comparison with hexane favors the azeotrope because 1) its lower boiling point, and 2) the simpler problem of dealing with the fines but this advantage may be offset by the cost of recovering the azeotrope from steam used in sparging the oil. The fines from the azeotrope settle very rapidly, in comparison with those obtained with hexane, to yield a clear final miscella.

The current costs of basic units for a conversion to azeotrope extraction with vibrating screen separators is given in Table VIII. Costs of installation and salvage value of existing screw presses and solvent extraction installations are not included in the table.

Evidently the capital investments in a conversion of existing prepress solvent and solvent plants to azeotrope plants to produce markedly superior meals and greater yields of oil of good quality should not be excessive.

In summary it may be noted that cottonseed meals that are equivalent or better than toasted soybean meal as protein supplements for broilers and swine are produced on extracting raw cottonseed flakes with water-saturated mixtures of acetone and hexane. Bleachable oils are obtained in yields significantly higher than those obtainable on extraction with hexane.

One process was worked out to adopt a solvent mixture to existing horizontal type basket extraction installations.

A second process was developed for extracting thin layers of flakes with the acetone-hexane-water azeotrope on a vibrating screen where quantitative extraction of oil (residual oil in meal less than 0.1 percent) is obtained in three minutes time or less. Costs of converting prepress solvent or solvent plants to azeotrope plants are estimated to be relatively low.

TABLE VIII.

Estimated Cost of Basic Units for a Three-Step Countercurrent Azeotrope Extraction Installation

Capacity	Size of vibrating screen	Estimation of cost for 3 vibrating screen units with stainless steel explosion-proof motors	Estimation of cost of screw conveyors	Estimation of pumps for miscellas	Estimation of price for unit light and rapid pressing of marcs	Total
10 tons per day	9'' radius	$ 2,400	$1,500	$ 150	$2,000	$6,050
28 tons per day	15'' radius	6,612	1,500	150	2,500	10,762
71 tons per day	24'' radius	10,080	1,800	200	3,000	15,200
140 tons per day	24'' radius	20,160	3,600	400	600	30,400
210 tons per day	24'' radius	30,240	4,400	600	9,000	45,600

REVIEW OF SU MIXED SOLVENT EXTRACTION: PROCESS AND PROPERTIES OF THE PRODUCTS

by

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Southern Utilization Research and Development Division
(Presented by Mr. Vix)

The chemical engineering development of the mixed solvent extraction process has followed a specific and direct path out of the vast amount of background technical data to the point where it is now a promising, commercially feasible and demonstrable process. Meal and oil products of the specified high quality as shown in Table I were obtained using pilot-plant equipment of the type and design used commercially in oilseed processing or related commercial operations.

Sufficient engineering and operating data have been obtained on the major processing steps --preparation of cottonseed meats and flakes, extraction, oil desolventizing and refining, meal desolventizing, and solvent recovery, control and remake--to warrant preliminary design of equipment and accessories to convert existing, direct, basket type hexane extraction plants to demonstrate the mixed solvent process on a commercial scale.

Gravimetric material balances on several pilot plant runs showed no loss or disappearance of oil or protein and an increased yield of refined oil per ton of cottonseed over conventional direct hexane extraction.

Preliminary estimates of installation and operating costs for a converted installation balanced against anticipated increased value of products indicate reasonable payout times to amortize the conversion costs.

TABLE I
MIXED SOLVENT EXTRACTION PRODUCT DATA

Meal:	
Free Gossypol	% 0.01 to 0.03
Total Gossypol	% 0.18 to 0.23
Lipids	% 0.30 to 0.50
EAF Lysine g./16g. N	4.30 to 4.35
Protein	% 50.1 minimum
Crude Miscella:	
Free Fatty Acid in Seed	% 1.2 - 1.5
Free Fatty Acid Equivalent in Crude	% 6.2
Neutral Oil	% 90.3
Refined Oil:	
Yield, % of Crude	90.8
Neutral Oil in Refined	% 99.4
Neutral Oil Yield	% 99.8
Refined Color	35Y/ 5.0 to 7.0 R
Bleached Color	20Y/ 2.0 to 2.5 R
Increased yield over conventional hexane extraction, Lbs.	9.3

The processing details given in the following sections are based upon a great many bench-scale tests and pilot plant runs in which the many processing variables were studied to correlate preparation, extraction, desolventizing, oil refining, and solvent recovery, control and remake.

The major steps of the process are shown in the simplified flow-sheet, Figure 1, and in greater process detail on the integrated flow-sheet, Figure 2.

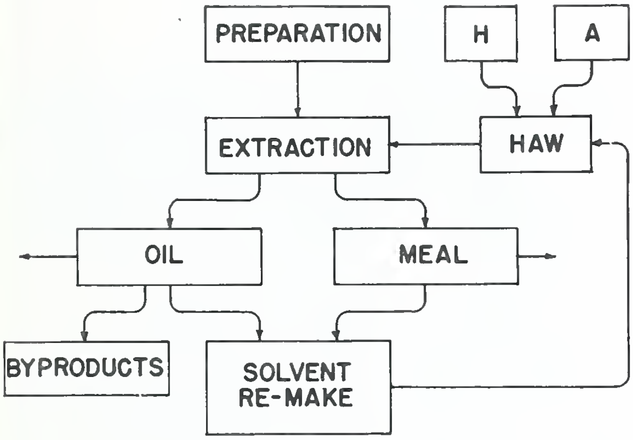

Figure 1

Preparation of cottonseed meats and flakes. The usual delintering and decorticating procedures are used. Emphasis is placed upon as complete a separation of hulls as possible to produce a final meal product containing 50% or more of protein. The meats are tempered in conventional equipment under mild conditions, 130° F. at 10.5 to 11.5% H_2O for 30 minutes and flaked to .006 in. thickness. The flakes are aspirated into a stream of warm air, heated to 140° F. for about 30 seconds, separated in a cyclone separator and cooled or aerated to about 100° F. These flakes are somewhat crisp, rather than flexible, and provide satisfactory percolation rates in the basket extractor.

Extraction. As shown on the detailed flow-sheet, extraction is in two countercurrent units. The first unit is a conventional basket extractor of the horizontal, rectangular or (probably also) of the deeper bed stationary or rotary basket types. Three stages of extraction should suffice and because commercial units contain 16 to 34 or more baskets a suitable combination can be easily devised to achieve the three stages. The second unit is a total immersion type of extractor, providing 15-20 minutes of countercurrent contact in the horizontal screw section followed by free suspension of the marc particles in the lower portion of the inclined drag part of the extractor. Before the inclined drag removes the marc from the extractor the marc passes over a perforated screen section which permits free solvent to drain from the marc.

Half of the fresh solvent enters the second unit just above the solvent level in the inclined section and the other half at the base of the drag after washing down any fines passing through the drainage screen. Half miscella overflows at the end of the horizontal screw section and most of the fines are removed in the settling compartment and returned to the marc discharge end of the horizontal unit.

Settled half miscella is the extracting solvent in the basket unit, usually with three countercurrent stages as shown. Part of the product miscella is refiltered through the baskets as shown.

Oil desolventizing and refining. Product miscella after passing through a polishing filter is concentrated to about 50% oil content and refined batch-wise, the soapstock being separated in a continuous, vapor-tight centrifuge. Clean separations are obtained without water washing, and after filtering with a small amount of filter aid, the refined miscella is further concentrated and stripped to yield a prime, bleachable refined oil, as shown in Table I.

Meal desolventizing. The extracted marc has been successfully desolventized in standard Schneckens type desolventizers without

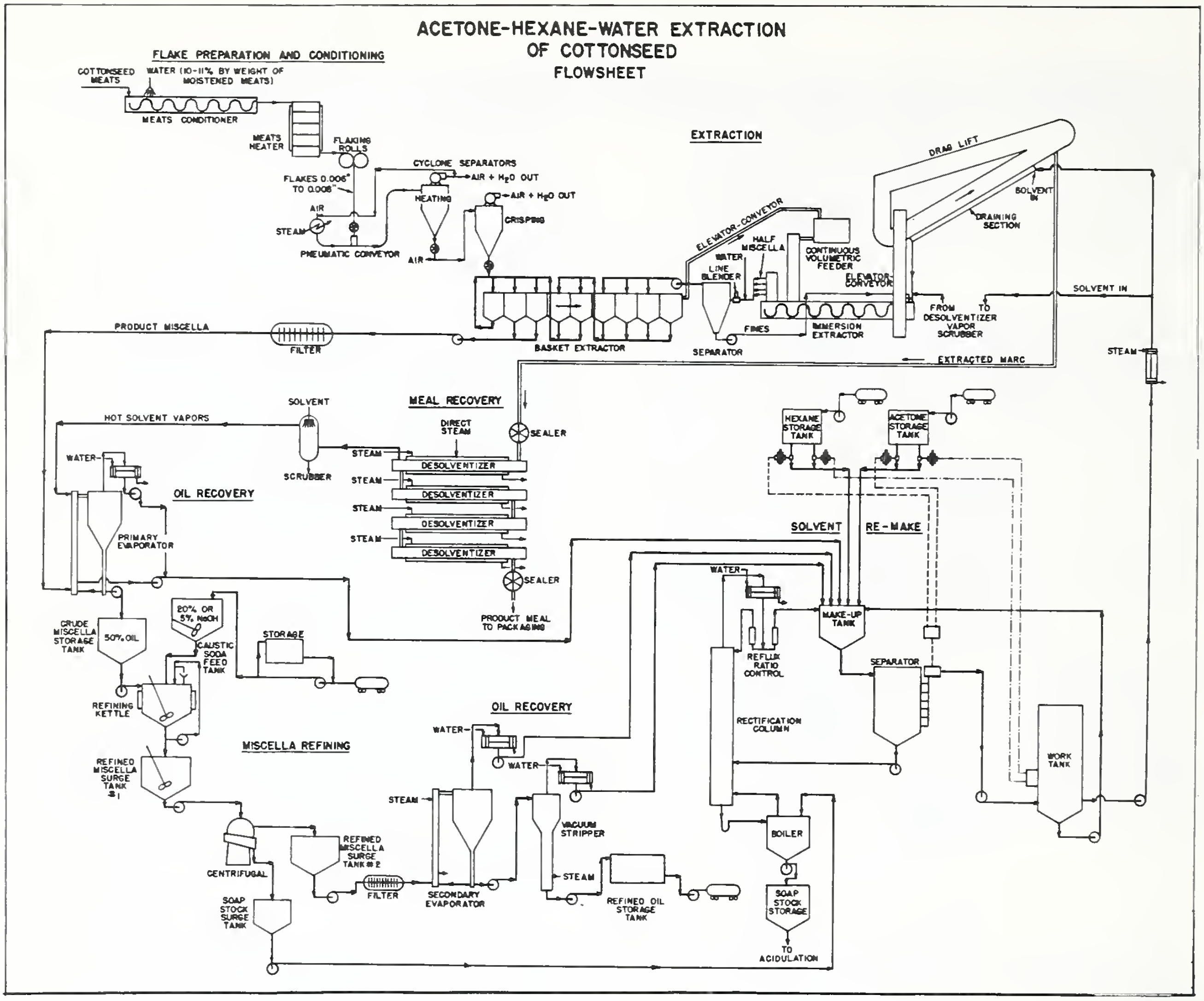

Figure 2

recourse to vacuum using some direct steam in the early stages. Maximum meal temperatures up to 225°F. show no adverse effects on the EAF Lysine analytical values. Typical meal product analyses are shown in Table I.

Solvent control and remake. All recovered solvent streams are returned to the make-up tank. Hexane and acetone to compensate for losses also enter this tank subject to control signals. Control instruments of the capacitance type monitor the upper, or saturated layer--calling for additional hexane or acetone for correction purposes as the case may be. The lower layer, containing the excess water picked up by the miscella during extraction and by the solvent during desolventizing passes to the rectifying column. Because the system is designed to operate on a saturated mixture of hexane, acetone, and water the excess water containing some acetone is removed by the centrifuge. The overhead product from the rectifying column may contain as much as 10% of water.

The work tank must be large enough to hold all the mixed solvent when the plant is shut down or laid by. The operating level is low in the tank and at a certain minimum point, controls function to admit hexane and acetone from supply tanks in proper proportion until the upper operating level is reached. The amounts of hexane and acetone admitted to maintain the operating level are equal to the operating solvent loss of the system.

The ternary diagram, Figure 3, depicts the system--hexane, acetone, and water. Saturated mixtures containing between 1 and 4% of water--points A and B, respectively, on Figure 3 have been used. The data reported was obtained with the composition 60% hexane, 39% acetone, and 1% water.

Material Balances

Extraction. Complete gravimetric material balances for several pilot-plant runs showed no significant imbalance for any significant element of the run. Data on a typical run in which 1, 206 pounds of flakes (7. 1% moisture) were processed, follow:

		Imbalance	
	lbs.	lbs.	%
Flakes	1, 120. 1	+5. 1	+0. 46
Total Extractable[1]	390. 6	+0. 7	+0. 18
Neutral Oil	368. 9	+1. 4	+0. 38
Total Gossypol	11. 59	-0. 73	-6. 3
Nitrogen	66. 4	+0. 4	+0. 6

All values on moisture-free basis.

Miscella refining. The following data on the same pilot-plant run were calculated from bench-scale refining tests:

	Total Extractables	Neutral Oil
Input, Crude Miscella	392. 0 lbs.	363. 5 lbs.
Output, Refined Oil	357. 0 lbs.	355. 0 lbs.
Yield	91. 1%	97. 7%

Preliminary Cost Estimates

In Table II preliminary estimated costs are given for additional equipment and its installation to modify existing basket-type

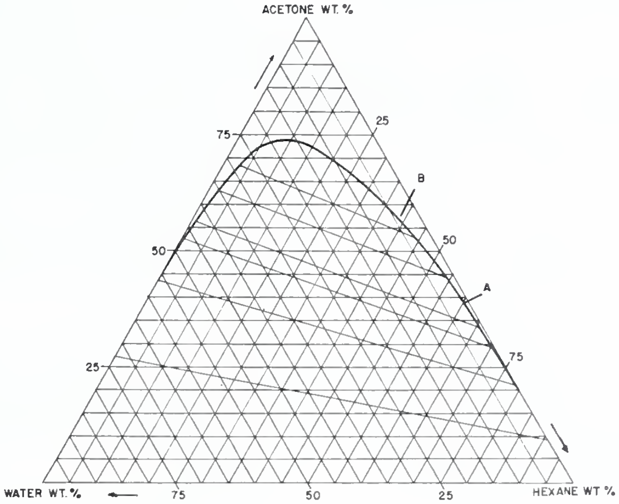

FIG. 3 TERNARY DIAGRAM - HEXANE, ACETONE, WATER

Figure 3. Ternary Diagram-Hexane, Acetone, Water

solvent-extraction plants for the use of the mixed solvent process. These estimates were made without benefit of actual quotations, but are believed to be reasonable, based upon the subject mill doing much of its own engineering, purchasing, and installation. The preliminary cost estimates are $200,000 for a 200 T/D plant and $300,000 for a 400 T/D plant.

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[1] Gossypol excluded.

To provide bases for calculating payout times, these two estimates and two larger estimates for each plant are included in Table II.

TABLE II

Conversion Costs

		200 T/D	400 T/D
Crisping Equipment		$15,000	$25,000
Extraction Equipment		40,000	60,000
Marc Desolventizing			
Oil Desolventizing and Refining		66,000	100,000
Solvent Control and Remake		30,000	45,000
		151,000	230,000
Misc. and Installation		49,000	70,000
Estimate No. 1	Total	200,000	300,000
Estimate No. 2	Total	300,000	400,000
Estimate No. 3	Total	400,000	500,000

In Tables III and IV, depreciation, interest, insurance, and maintenance, are combined with estimated per ton increased operating costs to get the total additional cost per ton of cottonseed for each size of plant and for each estimated modification cost.

These additional costs must be amortized from additional revenues derived from oil and meal products. Such data are shown in Table V. The refined oil price, normally quoted at New York, has been adjusted to Memphis by subtracting the freight (in tank cars) between the two points. Average values for July, August, and September 1963 were used. There may be as much as 20 pounds of lipid material and nine pounds of gossypol per ton of cottonseed present in the soap stock. A nominal value of 10 cents per ton of cottonseed has been assigned to this material.

It is believed that the combined increased value per ton of products might be in the range of $2.50 to $3.50 in the Valley, $3.00 to $4.00 in the Plains area, and $5.00 or more on the West Coast.

Payout times for these and other values may be noted from Figure 4, for a hypothetical 200 T/D plant and from Figure 5 for a 400 T/D plant. On the basis of the possible increased values of products stated above, the modification cost for a 200 T/D plant could be as much as $300,000 in the Valley and $400,000 in the Plains area or West Coast. Similarly for a 400 T/D plant, modification costs up to $500,000 might be amortized in a reasonable time in all areas.

The critical item is the increased value of products. If these values should be less than those indicated above, then the decision to convert to mixed solvent will need closer scrutiny.

The mixed solvent process is now developed sufficiently for serious consideration for commercial trial, especially in

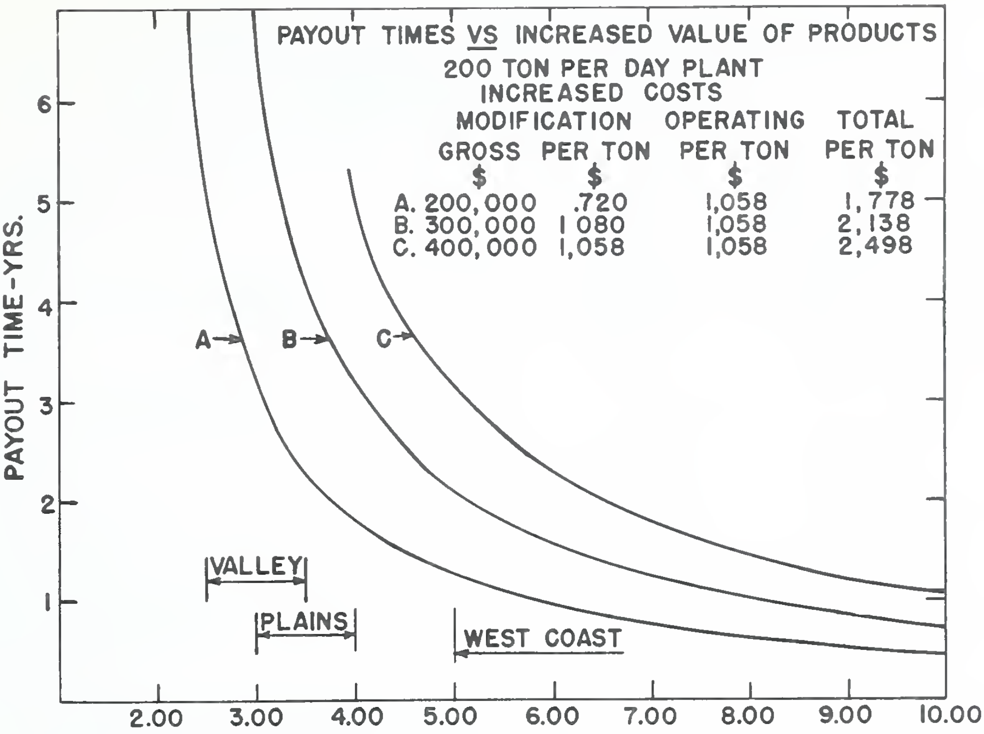

Figure 4. Increased Value of Products - $/Ton of C/S

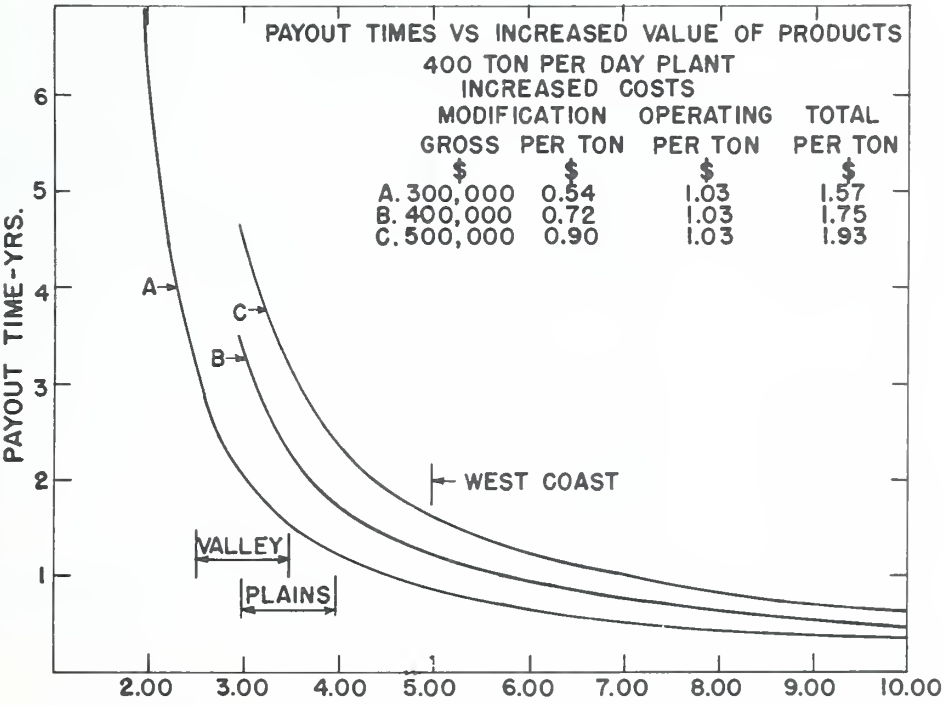

Figure 5. Increased Value of Products - $/Ton of C/S

TABLE III
INCREASED COSTS PER TON OF COTTONSEED
Size of Plant 200 Tons per day
250 Operating Days per year
Annual Capacity 50,000 tons

Cost to modify	$	200,000	300,000	400,000
Depreciation	10%	20,000	30,000	40,000
Interest	4%	8,000	12,000	16,000
Insurance and Maintenance	4%	8,000	12,000	16,000
Total		36,000	54,000	72,000
Per Ton of c/s	$	.720	1.080	1.440
Increased Operating Costs:				
Labor		.180	.180	.180
Electricity		.140	.140	.140
Steam		.500	.500	.500
Water		.100	.100	.100
Solvent Loss		.138	.138	.138
Total additional cost per ton of cottonseed		1.778	2.138	2.498

TABLE IV
INCREASED COSTS PER TON OF COTTONSEED
Size of Plant 400 Tons per day
250 Operating Days per year
Annual Capacity 100,000 tons

Cost to modify	$	300,000	400,000	500,000
Depreciation	10%	30,000	40,000	50,000
Interest	4%	12,000	16,000	20,000
Insurance and Maintenance	4%	12,000	16,000	20,000
Total		54,000	72,000	90,000
Per Ton of c/s	$	0.540	0.720	0.900
Increased Operating Costs:				
Labor		0.150	0.150	0.150
Electric Power		0.140	0.140	0.140
Steam		0.500	0.500	0.500
Water		0.100	0.100	0.100
Solvent Loss		0.138	0.138	0.138
Total additional cost per ton of cottonseed		1.568	1.748	1.928

TABLE V. Increased Value of Products

		Pounds per ton of cottonseed		
		Mixed Solvent Extraction		Conventional Direct Hexane Extraction
Flakes		1176		1176
Total extractables		380.8[1]		380.8[1]
Gossypol in flakes		10.9		10.9
Meal product (51.5% Protein)		785		785
Residual extractables in meal		5.5 (.7%)		9.8 (1.25%)
Crude oil		384.2[2]		371.0[1]
Refined oil	(8.93% loss)	350.0[3]	(8.2% loss)	340.7[4]
Av. value of crude[5] Memphis 10.3¢		-		38.20
Av. value of refined[5] Memphis 11.4¢		$39.90		38.90

		Over Conventional Refined[6]	Over Conventional Crude
Increased value of mixed solvent refined oil		$1.00	$1.70
Soapstock credit		.10	.10
	Total	1.10	1.80

Increased Value of Mixed Solvent Meal		Combined Increased Value of Product	
Per ton of meal	Per ton of cottonseed		
+$2.00	+$0.79	$1.89	$2.59
+ 5.00	+ 1.98	3.08	3.78
+10.00	+ 3.95	5.08	5.75
+20.00	+ 7.90	9.00	9.70

[1] Gossypol excluded

[2] Gossypol included

[3] By test

[4] Calculated: Ref. loss = 2.1 x FFA + 4.0

[5] For July, Aug., Sept., 1963

[6] If mill-site refining presently used.

areas highly dependent upon cottonseed as a source of protein concentrates.

In the event that residual lipids of about 1% in the meal product are acceptable, the secondary, total immersion extractor can probably be omitted. The use of mill-site miscella refining equipment is indicated because of the high gossypol content of the crude miscella. In spite of this, prime bleachable refined oils are produced.

DISCUSSION

L.A. Goldblatt: Thank you. Both presentations are now open for discussion. Are there any comments or questions?

J.A. Price: Dr. Frampton, you mentioned that the boiling point of this hexane was 62 to 78° C. and you called this typical. I don't think this is a typical hexane fraction -- in degrees Fahrenheit this would be 143.6 to 172.4. I think commonly they run closer to say 150 to 160. If this is true, is this the fraction which you are using in your mixed solvent system?

V.L. Frampton: The boiling point range that I cited is the boiling point range that we determine, and this boiling range determines which fraction we are working with. I don't know offhand what the boiling point of hexane per se is, but in our discussion we have referred to this fraction with a boiling range of 62 to 78° C. as hexane. Of course, it is a mixture, but the boiling point range that I reported is the boiling point range that we determined experimentally for this solvent.

Vacit Tümer: I would like to ask you whether there is a difference between this azeotropic mixture and the commercial hexane in the sense of explosion and danger.

V.L. Frampton: There may be a difference but I think perhaps the difference isn't too significant. In any case, one wants to avoid the hazard of explosion or fire.

J.A. Price: I think maybe I can help you out a little bit. I think if anything your mixture is probably a safer one than the straight hexane. I may have something further to add to this when my turn comes.

G.C. Cavanagh: I would like to ask Mr. Vix if he could give a little bit about some of the refining loss data. On Table I we see that the free fatty acids in the seed process was 1.2 to 1.3 percent. Then under the refined oil, the yield of refined oil from the crude is 90.8 percent. This would be equivalent, I believe, to a 9.2 refining loss. Vernon's slide showed that appreciably all of the oil was exhaustively extracted to zero residual oil. Is that correct?

V.L. Frampton: To which slide are you referring?

G.C. Cavanagh: I don't know precisely which one it was, but after one or two minutes, you showed zero residual oil.

V.L. Frampton: Essentially zero, yes.

G.C. Cavanagh: Okay, so we have then a 9.2 refining loss and a 1.2 to 1.5 fatty acid content. Am I interpreting this correctly?

H.L.E. Vix: I will ask for some help from my people and maybe from the oilseed people. We are taking out in addition to the free fatty acid, all of the gossypol-like pigments that are extracted. These are included in the free fatty acid equivalent in the crude. When we determine the free fatty acid in the crude, that includes the gossypol.

G.C. Cavanagh: Well, what would the total gossypol be, say one percent in addition to the free fatty acid? Might not we say that we have one percent of pigmentation?

E.A. Gastrock: Maybe as much as three percent in the oil.

H. L. E. Vix: I think it would be higher than just one percent.

G. C. Cavanagh: Well, I would point out that normally two to three times a fatty acid would be a normal refining loss, but this data would indicate that it is 6.13 to 6.17 refining loss based on the fatty acid. Maybe I am neglecting to take something into consideration, but taking into consideration the free fatty acid and the total gossypol what else might we consider?

H. L. E. Vix: Ed, I think you might want to make a comment on that.

E. A. Gastrock: If you look at Table I under Crude Miscella, it says free fatty acid in seed; 1.2 to 1.5. As all of you processors know, such a free fatty acid will increase slightly during the processing so that the free fatty acid in the extracted oil will be higher. Now, in addition to that, there is an amount of gossypol removed with the crude oil which may be as high as three percent of the crude oil. As the next line shows, we have an equivalent free fatty acid in the crude of 6.2, and that 6.2 equivalent gives us a refining loss of 9.2. This is what we get as the actual results.

G. C. Cavanagh: Well, this 6.2 is the figure that I can't reconcile.

E. A. Gastrock: You can't reconcile work on this type of miscella with any previous known miscella or crude oil.

L. A. Goldblatt: Our next paper is by a man whom we all know well by name and many of you know well in person. Unfortunately, I am not one of those who did know Dr. Vaccarino personally before today, but a man who has a process named after him doesn't need a lengthy introduction so I will simply present to you Dr. Vaccarino.

COMMENTS ON THE VACCARINO PROCESS

by
Carmelo Vaccarino
The G. & S. Vaccarino Company
Venetico Marina (Messina), Italy

You must excuse me at the beginning for my bad English, and I hope that you will appreciate not only the contents of my speech but also my courage to speak English. I thank you, Mr. Chairman, and also the organizers of this meeting for having given me the opportunity to present the results of our work. I also wish to thank for his kind, friendly collaboration, Mr. Clark, who visited our factory many months ago, who spent time in helping me in the preparation of my paper and who will help me, I hope, if I am unable to answer in English some questions. Last, but not least, I must thank Dr. Kapsiotis who for two years has been following what we are doing in Venetico, Messina; Dr. Kapsiotis went several times to our factory to take samples and gave us valuable suggestions to improve our process.

You have here a copy of the paper that I shall present. Some of the things in this paper are not complete. After this discussion I can give you some other information on this argument. But I would state that what I think is important in the matter I am here exposing to you, is that this process is not a laboratory process; it is a new system that has been operating for many years on an industrial scale. It is not the industrial scale of the United States, only 50 tons of seed per day, but for us in Europe it is not a pilot plant. By operating and improving this plant in the last few years we now have information that puts us in position to project larger plants operating continuously; one of the possible solutions, we hope, to the problem of producing a cottonseed meal to be used both for human consumption and for feeding monogastric animals. To explain, in our factory in Sicily at this moment we process flakes obtained by screw prepressing, and by this system we obtain a meal that is regularly sold on the Italian market for feeding chickens and pigs. In these last years our attention has been drawn to the possibility of using such a meal for human consumption in the underdeveloped countries. And here again I have to remember Dr. Kapsiotis who was the first to point this problem out to us.

Of course it is not the same thing to produce a meal for animals and food for human consumption. Our first attempts were to demonstrate the possibility of processing the cottonseed directly without prepressing, I mean processing by extraction with acetone. This is not the place to describe the difficulties we encountered, but what I can state, is that for a few months now we are sure that all difficulties are solved. I can also announce to you that a company to be established in the next few weeks in Europe has the aim to exploit this new system, utilizing the experience gained by the practical processing of our plant in Italy. As I told you, the difficulties arising from direct acetone extraction are now solved, and we could project without delay small installations to process from 50 to 80 tons of cottonseed per day by direct extraction, to produce a meal for human consumption. For plants of larger size the main problem is to choose a more suitable continuous extractor from the types existing on the market. In the paper that you have there are shown some analytical results of our meal. These analyses were made here in the United States by Texas A&M University and by private laboratories. There are also reported analyses made in Italy and the results of biological trials carried out on chickens and on pigs. I have to explain to you that these trials were carried out, above all, to give us the certainty that our meal was not toxic. Perhaps the composition of some of our feeding mixtures could be criticized, but I hope that results will soon be available of more complete biological experiments which could give a definite conclusion about this argument. Mr. Clark will now present to you the results of the process and his comments. I will afterward be glad to answer any questions that you pose to me. Thank you.

THE VACCARINO PROCESS FOR THE EXTRACTION OF COTTONSEED OIL BY ACETONE

by
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Venetico Marina (Messina), Italy

(Presented by Stanley P. Clark)

The process has been employed regularly for about seven years in a plant in Sicily working 50 to 60 tons of cottonseed per day. Information about it has been given in several papers and articles published in these last years. (1, 2, 3, 4). At the same time modifications and improvements have been made to the original process for simplification and safety and to reduce the processing cost.

As the results obtained are now considered satisfactory, a developing program is being undertaken to extend this process to countries which are much more interested than Italy in cottonseed processing. The first step of this program is the projection of a plant of higher capacity and with continuous functioning, which would guarantee the high quality of the products already achieved and lower working costs.

The Vaccarino Cottonseed Meal Obtained by Prepressing and Extraction

The existing plant in Venetico (Messina) was initially designed to process the flakes obtained by decorticated cottonseed prepressing, in order to obtain a meal which would be suitable for unlimited feeding of chickens, laying hens, and pigs.

This aim has been reached completely and the cottonseed meal obtained by this process is now sold in Italy at the same price as soya bean meal of equal protein content. In fact, it has been confirmed practically that by adding Vaccarino cottonseed meal to the soya bean meal used in a poultry mixture, an improvement of the nutritional value is obtained that results in a faster increase of weight and better plumage. At the same time, no negative aftereffects have been noted (e. g. stress, canabalism, illnesses, etc.).

The results can be attributed to the degossypolizing obtained by the solvent properties of acetone and the low temperature at which the processing is done, causing not only the elimination of the toxicity but also a more complete retention of availability of the essential amino-acids contained in the cottonseed meal and particularly of the lysine, which, as is well-known, tends to bind itself with the gossypol and is very sensitive to the high temperature.

Samples of the prepress-solvent meal, after enrichment in protein content by screening out hulls, were analyzed in 1961 on behalf of the Food and Agriculture Organization of the United Nations by the Barrow-Agee Laboratories of Memphis, and by Texas A. & M. University, obtaining the results shown in Table I, first and second columns.

The Vaccarino Cottonseed Meal Obtained by Direct Extraction from the Decorticated Cottonseed and its Use for Human Consumption

As a result of the interest shown by the FAO in the Vaccarino process, in the last two years the possibility has been studied of producing a cottonseed meal of a still better quality which could be used for human consumption, particularly in the developing countries. To achieve this, a system of direct extraction of the decorticated cottonseed has been applied, eliminating the prepressing (which even if conducted at a low pressure always causes some heating of the seed and therefore a loss of lysine). The

TABLE I. Analysis of Meals in 1961

	Prepress-Solvent Meals		Direct Extracted Meals	
	FAO 234-1	FAO 234-2	FAO 234-3	FAO 234-4
Moisture	7.40%	4.14%	4.24%	4.48%
Protein	50.19%	56.13%	58.56%	54.06%
Fat	1.55%	0.50%	1.30%	0.95%
Free fatty acid in extracted fat	24.9 %	24.2 %	10.6 %	24.1 %
Crude fiber	4.50%	4.20%	4.03%	4.33%
Free gossypol	0.03%	0.03%	0.01%	0.03%
Total gossypol	0.54%	0.53%	0.36%	0.26%
Available lysine:				
% of sample	1.54	1.74	1.43	1.73
% of protein	3.14	3.24	2.54	3.36

same care has been taken in the desolventizing of the exhausted meal, which is to be carried out without exceeding 70°C. (158°F.).

The first industrial production met with some technological difficulties caused by the high quantity of oil to be extracted and by the moisture in the seed, which dilutes the acetone, thus increasing the required quantity of the latter.

Nevertheless the results of the analyses made in the United States in 1961 appeared very encouraging. These results, which are contained in Table I, columns 3 and 4, confirmed that by this way it was possible to obtain a cottonseed meal with still lower total gossypol content. The lysine content in column 3 appeared, on the contrary, unsatisfying; this was caused by the system at that time used for desolventizing which did not assure a sufficiently low temperature.

In 1962 and 1963 other samples of meal of the same type were analyzed by U. S. laboratories, at the request of FAO and UNICEF, obtaining the results shown in Table II. (5, 6, 7).

At the same time, biological trials were carried out to determine the effect of the introduction of this type of cottonseed meal in mixtures employed to feed different animals. In Figure 1 the results are shown of feeding trials on rats with Vaccarino direct-extracted cottonseed meal, used at different levels, together with soya bean meal (8). It is possible to see that the best results seem to be obtained with a mixture at the ratio 50 percent cottonseed meal and 50 percent soya bean meal. This mixture appears to be considerably superior to a similar mixture containing only soya bean meal.

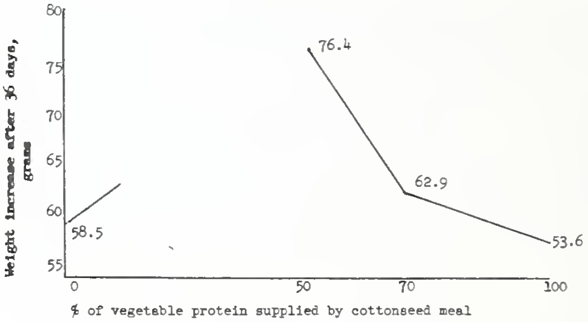


Figure 1. Results of feeding trials on rats with Vaccarino direct extracted cottonseed meal in combination with soya bean meal. (0% cottonseed meal is 100% soya bean meal.)

Trials with pigs were conducted by the Zootechnic Experimental Station of Monterotondo (9) using two mixtures: (A) containing 36 percent of Vaccarino cottonseed meal and (B) 30 percent of soya bean meal and 4 percent of meat meal. The figures in Table III summarize the results of these trials.

These figures indicate that it is possible to feed pigs with Vaccarino cottonseed meal, obtaining more favorable results than with a mixture of soya bean and meat-meal (weight increase 207 percent against 192 percent). The feed conversion also gave results lower for the cottonseed meal diet than for the soya bean meal diet.

To determine the influence of cottonseed meal on chicken feeding, several experiments were conducted in 1962 directly by the Vaccarino Co. (groups 1-5). Other chicken feeding trials were carried out very recently by the Istituto di Zootecnia Universita Cattolica del S. Cuore di Piacenza. (groups 6-9). The results of both of these series of tests are summarized in Table IV.

TABLE II. Analysis of Meals in 1962 and 1963

	A	B	C
Moisture	8.54	-	7.14
Protein	54.81	-	-
Fat	0.50	-	-
Free fatty acids, in extracted fat	60.97	-	-
Crude fiber	4.1	-	-
Ash	6.15	-	-
Acid insoluble ash	0.10	-	-
Soluble nitrogen in (0.02N NaOH)	86.89	-	-
Total gossypol	0.28	-	0.32
Free gossypol	0.03	-	0.05
Available lysine, percent of protein		4.09	4.22

TABLE III. Results of Feeding Trials on Pigs

	Group A			Group B		
	1	2	3	4	5	6
Final weight increase	30	27	23.6	27	27	22
Average weight increase	207%			192%		
Feed consumed Kg.	85	74.5	78.5	79.5	89.5	69.5
Feed conversion	2.84	2.76	3.32	2.94	3.32	3.15

The best results in both series were given by rations containing both Vaccarino cottonseed meal and soybean meal. Comparing all the results of the experiments described, it is possible to conclude that employing Vaccarino direct-extracted cottonseed meal at a ratio varying from 30 to 50 percent of the total proteic foodstuffs, it is possible to obtain the same or a superior weight gain than using only soya bean meal. Furthermore, the fact is to be emphasized that the cottonseed meal used in all these experiments was prepared by processing unselected cottonseed of common quality, which had been stored for several months.

It seems also likely that the quality of this meal could be notably improved if it will be produced in a specially designed continuous plant, where the temperature control could be effected much more strictly than in the actual discontinuous plant. Finally is to be considered that the conditions of working (as temperature, moisture length of final heating) are not yet determined to obtain "the best" cottonseed meal. For instance,

TABLE IV. Results of Feeding Trials on Chickens

Group	Protein sources, % of ration			Final weight grams
	Vaccarino CS meal, 52% pr.	Soybean meal, 45% pr.	Fish meal 50% pr.	
1	25	-	10	1020
2	12.5	12.5	10	1150
3	12	15	3	1130
4	8	5	7	900
5	"Chifa," commercial mixture			1000
6	-	26	-	1328
7	13	13	-	1311
8	8	18	-	1376
9	26		-	1255

Rations for groups 3 and 4 included 1.5% dried whey and 3.5% alfalfa meal which also contain protein.

by the results of a very recent study carried out in the "S. Cuore" University of Piacenza, it seems that a final toasting of the Vaccarino degossypolized meal improves considerably the nutritive value of the same in spite of the apparent worsening of its analytical characteristics (i.e. lysine content).

Outline of the Direct Extraction Processing and of the Working Costs

The decorticated cottonseed is ground in a corrugated mill and afterwards flaked to obtain flakes of thickness 0.2 to 0.4 mm. (0.008 to 0.016 inches) which are extracted by rectified acetone having a concentration of 95-97 percent. The extraction is done at a temperature of about 50°C. and produces a perfectly homogenous miscella containing 23-26 percent of oil.

With the industrial system followed up to this moment, which uses rotative extractors operating discontinuously, four washes are necessary, of which the last is with pure acetone and the others are miscellas successively more concentrated. The first wash miscella is utilized in the second step of the process, which is oil refining. To achieve this the miscella is concentrated to bring the ratio oil-acetone to about 1:1.2, and afterwards continuously neutralized, following the process already described (4). The oily phase obtained after the centrifuging is washed with hot water and centrifuged again in a second centrifugal separator, to eliminate the soap traces present in the neutralized oil. In this way a neutralized oil is obtained, retaining only a small quantity of acetone which is removed by a stripping with direct steam. The water-phase is heated to about 105°C to eliminate all the acetone contained in it; a great part of the water solution so obtained is recycled and utilized for the dilution after neutralization; the residue, which contains more concentrated soap, is discharged.

Obviously, in a plant working discontinuously in part, it is not always possible to use heat exchange to recuperate a part of the heat employed in the processing. There is no difficulty in achieving this in a plant working continuously. In this case it is easy to demonstrate, that the steam consumption required by the acetone process is not much higher than that required by a modern hexane plant.

Regarding this argument, the first observation that could be made is that acetone, having a heat of vaporization higher than hexane (124 cal/Kg. against 89 cal/Kg.) requires for its distillation a notably higher quantity of steam. This is generally true, but if one considers that in the case of acetone the condensation heat is greater to the same extent, it follows that if the condensation heats of acetone vapors produced by the rectifying column, by the miscella concentrator and by the meal desolventizer are recovered through heat exchange, in practice the steam required by the acetone distillation can be notably reduced. This heat exchange, the only obstacle to which is the low acetone condensation temperature of 59° C. (138° F.) will be achieved in the continuously working plant that is now in projection, through compression of the pure acetone vapors in the plant, in order to increase their condensation temperature and thus allow the utilization of these vapors as a heating fluid (see flow-chart).

It is also possible to recover nearly all of the heat employed for heating the water required in the process; in fact, a great part of the soapy water is recycled, and the remaining part exchanges its heat with the fresh acetone used for the extraction.

Particular care must be taken with the exhausted meal desolventizing to prevent the temperature rising above 70°-80°C. This is necessary to preserve the lysine content. To achieve this aim, the desolventizing will be effected in two steps, the first at ordinary pressure, (the presence of a high quantity of acetone prevents excessive temperature rise) the second, under vacuum in which the acetone is completely removed by a jet of direct steam. For both steps the indirect heating is obtained by using the compressed acetone vapors which condense at about 85-90°C, so eliminating all risk of overheating.

Concerning the rectifying column, the last improvements have very much reduced its load. In fact, the acetone vapors produced by the miscella concentration and by the first step of meal desolventizing do not need to be rectified. Thus, only the vapors and the solutions obtained by the second step of the desolventizing (where direct steam is injected), by the distilling of the water-phase, and by the oil stripping, are conducted to the rectifying column. For this work a column of small dimensions is required.

By utilizing the specific drying and other preparation techniques developed for this process, extraction of oil (and gossypol) is more rapid with acetone than with hexane in a normal hexane process. Percolation type of extractors are preferred, which operate with lower solvent requirements.

The concentration of oil in acetone miscella is about the same as in hexane miscella. In many other ways the acetone and hexane extraction processes are so much alike that only small changes would be necessary to modify existing hexane extraction plants to operate on acetone.

Increased Costs Using the Vaccarino Process

Operating Costs

In Table V the principal processing data are indicated relating to the Vaccarino-Process and to a modern hexane plant operating in Europe. The plant is one processing 400 tons of cottonseed per day. All tonnage figures are metric tons of 1000 kg. or 2200 lbs. per ton. The costs are calculated in U. S. dollars, considering the following average unitary costs in the USA:

Steam	$ 3.00	per ton
Electric Power	$ 0.012	per kw.-hr.
Cooling Water	$ 15.00	per 1,000 cm
Acetone	$143.00	per ton
Hexane	$ 62.00	per ton
Fuel-oil	$ 16.00	per ton

The additional operating cost per ton of seed for the Vaccarino plant is: $1.21 x 0.57 = about $0.69.

Cost to Modify an Existing Hexane Plant

The plant is one processing 400 tons of cottonseed per day. This cost is estimated at $300,000. The additional annual costs for depreciation, interest, insurance, and maintenance are:

Depreciation	10%	$ 30,000
Interest	4%	$ 12,000
Insurance and maintenance	4%	$ 12,000
Total		$ 54,000

Considering a total annual capacity of 100,000 tons of cottonseed to be processed, the additional cost per ton of seed is $0.54.

Total Additional Costs Using the Vaccarino Process

Operating cost	$0.69	per ton of cottonseed processed
Plant cost	$0.54	per ton of cottonseed processed
Total	$1.23	per ton of cottonseed processed

Increased Value of Products

Higher Yield in Refined Oil

The neutralization loss in the Vaccarino Process may be estimated, for oils of average quality, as about 1.2-1.6 times the chromatographic loss of the crude oil. As the neutralization loss for the batch neutralizing system is 2-2.5 and for the systems employing centrifugal separators is 1.4-1.8, the higher yields allowed by the Vaccarino system are calculated below:

Chromatographic loss of crude oil	3	5
Loss by batch process	6.75%	11.2%
Loss by centrif. process	4.8 %	8.0%
Loss by Vaccarino process	4.2 %	7.0%

If 170 Kg. of crude oil are extracted by 1 ton of processed cottonseed, the higher yields of refined oil per ton of cottonseed allowed by the Vaccarino process are:

Chromatographic loss	3		5	
	Amount	Value*	Amount	Value
Higher yield ref. to batch process	4.3 Kg.	$1.07	7.1 Kg.	$1.78
Higher yield ref. to centrif. process	1.0 Kg.	$0.25	1.7 Kg.	$0.42

*Value of neutralized oil at $0.25 per Kg.

Higher Value of the Meal

The higher value of the meal depends on the utilization of the same; it can be estimated to be varying from $4 to 15 per ton (poultry feeding or human nutrition). The profit allowed by the Vaccarino system for these two extreme cases, considering a yield of 370 Kg. of meal (44-46 percent protein) per ton of seed (to be eventually enriched to 52-56 percent protein) is:

	Poultry feeding	Human alimentation
Higher value of the meal per ton of seed	$1.48	$5.55

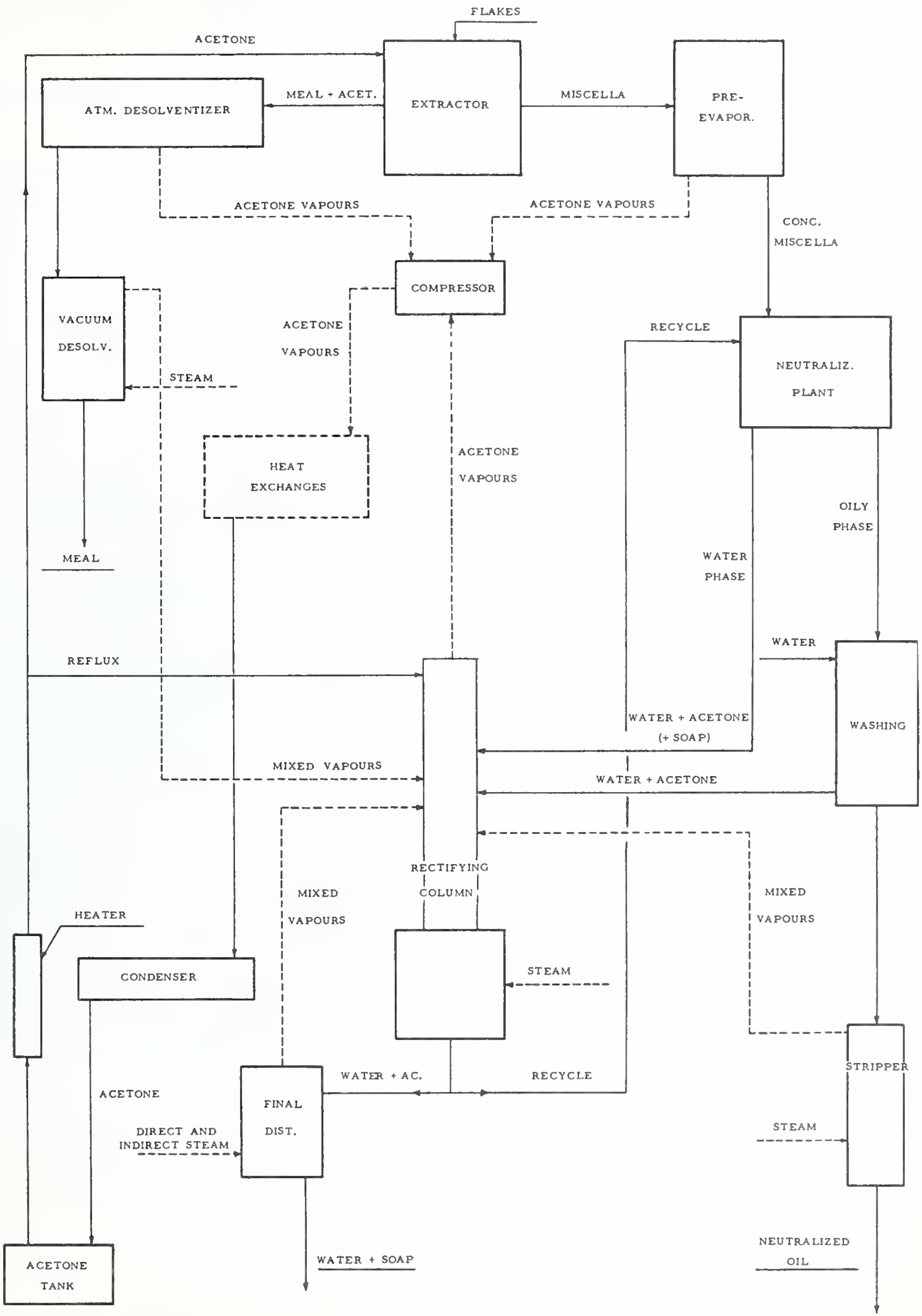
Summary

Against an additional cost of about $1.23 per ton of processed cottonseed, the Vaccarino system allows the following profits per ton of cottonseed:

For higher yield of neutralized oil:	from	$0.25	to	$1.78
For higher quality of the meal:	from	$1.48	to	$5.55
Total	from	$1.73	to	$7.33

In the calculations above, the plant and operating costs of the conventional batch or centrifugal neutralization systems were not considered. Adding these costs, the profit allowed by the Vaccarino process would be much increased.

SIMPLIFIED FLOW CHART OF THE VACCARINO PROCESS



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Further Comments by Carmelo Vaccarino

It is evident that before beginning the projection of new plants it has been necessary to have a complete engineering process. In the short time we have available it is impossible for me to give you extensive data on this item, but I will be glad to meet privately with anyone who desires more details. I am ready to show the flow sheet of the process and general calculations to demonstrate the possibilities that it offers, with operating costs not much higher than costs of traditional installations.

The figures that I quote relate to the new continuous plant that we are projecting. The steam, electric power and cooling water requirements are to be considered as certain, but I am also sure that they can be improved. For instance, we foresee a steam requirement of 280 kilograms per ton of flakes processed. This figure, as you understand, is a little better than the figures given by the more modern extraction plants using hexane. For the plants designed ten years ago the steam requirement was of 500 kilograms per ton of flakes. Regarding the electric power we need about 80 kw.-hr. per ton of flakes processed, that is higher than the figures given by the traditional plants. The amount of water is 7-8 cubic meters per ton of flakes. Lastly, we require an additional consumption of about 16 kilograms of fuel oil per ton flakes processed.

In the calculation of processing costs it is necessary, of course, to consider the difference in price between acetone and hexane. In some countries this difference is not high. For instance, in Italy acetone and hexane have the same price. The price of acetone is generally lowering in all countries as its production exceeds the requirements of industry. In Europe now it is half of what it was four years ago. I have heard that also in the United States the acetone price is lowering; now it is 2.3 times the price of hexane in weight. Considering a loss of solvent of 5-6 Kgs. per ton of processed flakes, this means by actual prices in the United States, an additional cost of about 55¢. This is not very high. This is the general information that I can give you. As I said before, I am at your disposition for giving you further details. Thank you.

REVIEW OF NEW SOLVENTS FOR OIL EXTRACTION AND MEAL PURIFICATION

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This paper reviews the customary commercial extraction solvents together with some interesting alternatives. For convenience, solvents for extracting dry materials and solvents for wet materials are discussed separately. Both groups of solvents have the same requirements except that solvents for wet materials also need to be efficient at water removal. For dry materials hexane has been the industry standard. Recent research has been on solvent mixtures since no individual solvent appears able to compete with or displace hexane. The only major drawback of hexane is its flammability.

The azeotropic extraction of wet materials has been accomplished successfully with trichloroethylene and dichloroethane. Commercial heptane appears somewhat more efficient and economical. Some new hydrocarbon fractions exhibit good azeotropic properties but, like hexane, they are flammable and must be handled accordingly.

Introduction

Following customary procedure of some papers, I will first indicate the topics that will not be discussed. This paper will not concern the solvent extraction of nonfood materials such as rosin, waxes, greases, etc. However, many of the solvents used for extracting these materials are also used for foods. Also it will not cover the mixed solvent system used by the Southern Utilization Division (11) nor the acetone-based process (6) of Mr. Vaccarino. As processes, these have already been adequately covered today, but I will comment on the individual solvents themselves. Further, this paper will not concern itself only with cottonseed, but with other food materials as well. However, some of the discussion on other materials will be pertinent to the cottonseed situation.

In short, what I do plan to review today are the solvents which have been or can be used to extract food materials, and their properties. To implement this, I have divided the solvents under study into two main categories: 1) solvents for extracting dry materials; namely, those with less than about 10% water and 2) solvents for wet materials; greater than 10% water. The need for some classification is evident from Table I which shows that extraction solvents are found in almost every class and sub-class of the solvent family. And these are but a few examples of the more commonly used extraction solvents. The solvent listing given here would have been even longer if mixtures of solvents such as the hexane-acetone-water mixed solvent just discussed by Dr. Frampton had been included. The asterisk notation on hexane indicates a problem basic to this well known solvent which will be discussed later in the paper.

TABLE I. Extraction Solvents

Class	Solvent	Material Extracted
• Hydrocarbon		
-Aliphatic		
Straight chain	Hexane*	Cottonseed, Soybean
Branched chain	Isohexane	Pyrethrum
-Aromatic	Benzene	Soybean
• Chlorine-containing		
-Unsaturated	Trichloroethylene	Soybean, Olives
-Saturated	Dichloroethane	Fish
• Oxygen-containing		
-Alcohol	Ethyl Alcohol	Cottonseed
-Ether	Ethyl Ether	(Laboratory extractions)
-Ester	Ethyl Acetate	Fish
-Ketone	Acetone	Cottonseed
-Aldehyde	Furfural	Cottonseed (oil)
• Sulfur-containing	Carbon Disulfide	Olives

*Mostly n-Hexane

Discussion

As just stated, it will be useful to discuss the solvents for wet and dry systems separately.

Solvents for Extraction of Dry Materials

First, it will be of value to examine the characteristics which are most desired in an extraction solvent. Table II gives a fairly complete listing of these. They will be discussed more or less in the order in which they appear although this order does not constitute necessarily their order of importance. A desirable extraction solvent is:

Oil miscible - If any single property of a solvent is the most important, it must be oil miscibility. Obviously if the oil is not soluble in the solvent, there would be no extraction. However, in testing the solubility of a new solvent, one must not be misled by immiscibility at room temperature. Elevated temperatures are the rule in solvent extractions and this is where the oil must be appreciably soluble in the solvent.

TABLE II. Desirable Solvent Characteristics for Extraction of Relatively Dry Materials

1. Oil Miscible
2. Selective
3. Stable
4. Unreactive
5. Low specific heat
6. Low latent heat
7. Low boiling
8. Narrow boiling
9. Low viscosity
10. Low specific gravity
11. Noncorrosive
12. Nontoxic
    - inhaled
    - ingested
13. Nonflammable
14. Impurity free
    - volatile
    - non-volatile
15. Inexpensive
16. Available
17. Water insoluble

As a matter of fact, a solvent having high solubility at elevated temperature and low or no solubility at room temperature would be desirable since separation of oil from solvent might occur at the low temperature, thereby reducing the solvent stripping requirement. The lower alcohols sometimes exhibit this characteristic with vegetable oils. The hydrocarbons, chlorinated hydrocarbons and chlorinated ethers are miscible at all temperatures.

Selective - Selectivity of the solvent is also a very important characteristic and can result in the choice of one solvent over another. While it is important for a solvent to efficiently extract the triglyceride fraction of oil, it is generally not desirable to extract the phospholipids, fatty acids, and other color bodies. Complete extraction, as you know, places a greater burden on the oil refiner to remove the extraneous materials carried into the oil. So even though trichloroethylene and carbon disulfide extract more oil from olives than hexane, 95% of the olive extraction plants in Italy use hexane. Benzene also is a potent extractant. For this and toxicity reasons it is kept at low levels in solvent hexane where it occurs as an impurity.

Another selectivity problem is well-known to this group -- the extraction of toxic gossypol from cottonseed. Hexane alone does not completely remove gossypol but the remainder in the meal can be deactivated by heat. The mixed solvent system acetone-hexane-water does remove gossypol but extracts other materials which add to the refining burden. By "heat fixing" the meal the problem seems to be farily well under control in the United States. (3) In addition, the development of the gossypol-free strain of cottonseed would seem to be a final solution to the problem. In certain areas outside the U. S., the gossypol situation is almost reversed because only the cottonseed oil is used as food. The meal is used as fuel. In this situation hexane with a very low naphthene content is used because more of the gossypol is left in the meal. Solvent selectivity is becoming more important because of the increasing need that both products be merchantable.

Some experimental data on solvent miscibility and selectivity is given in the next few tables. Table III compares the effects of three hexanes and benzene in extractions on soybean and cottonseed. (4) Extraction completeness and rate (see later section) are partially related to solvent solubility of miscibility, and the oil quality as measured by free fatty acids and color is related to the selectivity. On the average, in soybean extraction, the hexanes appeared to extract more slowly but to give better oil quality than benzene. Both groups were equivalent in completeness of extraction. With cottonseed, there was no difference in extraction completeness or rate between the hexanes and benzene. Overall, the benzene-extracted oil was poorer in quality.

In Table IV, five solvents are compared with respect to their effectiveness in cottonseed extraction. (5) Based on overall oil yield and quality, the order of decreasing solvent desirability was hexane > benzene > ether=butanone > acetone. This kind of solvent comparison shows again that benzene is poorer than hexane as a solvent, but that other solvents are poorer than benzene. Hexane and benzene gave the least yield of oil but the most neutral oil. This is an excellent example of the inverse relationship between crude oil yield and oil quality.

Solvent composition also has effects on selectivity. As you know some commercial solvents are not single compounds. Commercial hexane is a mixture of many C_5-C_7 isomers and includes aliphatic compounds, naphthenes and aromatics. Typical compositions for some commercial hexanes are given on Table V (Hexanes A, B and C were used in Table III). Hexanes like A generally are not used for extraction purposes. The n-hexane content of commercial hexanes can vary from nearly 100% down to less than 50%. The other isomers vary accordingly. This variation in isomer distribution obviously has some effect on the extraction selectivity and efficiency. For example, it is known that hexanes containing low percentages of naphthenes are less efficient in their extraction of gossypol from cottonseed. Table VI shows that based on completeness of extraction and the quality of oil obtained, the isomeric

TABLE III. Solvent Comparisons in Soybean and Cottonseed Extractions

	Solvents			
	Hexane			Benzene
Soybeans	A	B	C	
Extraction Completeness	2	1	1	2
Extraction Rate	3	2	2	1
Oil Quality				
-FFA	1	3	3	2
-Color	1	2	2	3
Cottonseed				
Extraction Completeness	1	1	1	1
Extraction Rate	1	1	1	1
Oil Quality				
-FFA	1	2	2	1
-Color	3	1	2	4

1 = Best
Conditions: Reverse Flow Extraction in Glass, 135-140°F.

TABLE IV. Solvent Comparisons in Cottonseed Extraction

	Hexane	Benzene	Ether	Acetone	Butanone
Oil Yield	4	5	2	3	1
Color, Crude Oil	1	2	3	5	4
Fatty Acid Material, Wt. %	1	3	5	4	2
Refining Loss, %	1	2	3	5	4
Gossypol Type Material, %	1	2	3	4	5
Neutral Oil, Wt. %	1	2	3	5	4

1 = Best
Conditions: Seed flakes all identical in preparation; SURDD pilot plant batch extractor; other conditions held as close as possible.

TABLE V. Typical Composition of Commercial Hexanes

	A	B	C	D
2, 2-Dimethylbutane	0.0	0.3	0.0	tr.
Cyclopentane	- -	- -	- -	tr.
2, 3-Dimethylbutane	0.0	0.0	0.3	16.5
2-Methylpentane	0.0	3.0	5.4	
3-Methylpentane	0.0		11.3	16.6
n-Hexane	99.5	88.4	59.7	46.2
Methylcyclopentane	0.0	7.6	22.4	18.6
2, 4-Dimethylpentane	0.0	0.7	0.0	0.1
Cyclohexane	0.4	0.0	0.9	1.8
Benzene	<0.001	<0.001	0.0	0.2

methylpentanes are superior to commercial extraction hexane. (1) However, the article reporting this data conclude that these isomers are too expensive for use as extraction solvents. Another study (2) of the selective effect of isomers on cottonseed is given in Figure 1. Use is made of a term called "quality-efficiency solvent rating" (QER). This was obtained by weighting the oil yield, refining loss and color factors and combining these into a single equation. A plot of quality efficiency rating against boiling point shows an advantage for 2-methyl and 3-methylpentane. All of the other isomers found in commercial extraction hexane fell below the QER rating of the whole commercial grade solvent itself. It is interesting to note that n-hexane rates poorer in QER than the total solvent and thus there seems to be no incentive to maximize it in extraction solvent. In this type of analysis, benzene has the poorest rating which is good reason to keep it to a minimum.

Stable - Extraction solvents should be stable to heat, light and water. Solvent recycle is a necessary procedure, and solvent must be able to withstand repeated vaporizing and condensing. Stability is needed not only to conserve solvent but to prevent contamination of the meal and oil with the decomposition products.

Unreactive - Extraction solvents must be unreactive physically, as well as chemically. Chemical reaction between trichloroethylene and cysteine in soybean meal was thought to be the cause of cattle deaths some years ago. (3) Alcohols because of their polar and hydrogen bonding nature absorb strongly on most solid residues and are difficult to recover. The unreactive, nonpolar hydrocarbons do not have this difficulty. In addition to nonreactivity with the material to be extracted, solvents must be unreactive to the other chemicals used in processing. For example, they must be stable or unreactive to water, steam, and various metals which they are bound to contact.

Easily Recovered - The solvent recovery part of an extraction plant can amount to 75% of total investment. Solvent properties such as specific heat, latent heat, boiling point, viscosity, specific gravity, surface tension, and amount of solvent required can greatly affect the size of this investment. Basically, the amount of solvent is adjusted to give a suitable extraction rate which is controlled by the expression, specific gravity x surface tension ÷ viscosity. (7) With the quantity of solvent fixed it is important that the solvent possess a low specific heat, a low latent heat of vaporization, and a low boiling point. This combination of terms determines the sensible heat required to reach the boiling point plus the heat of vaporization per unit volume of solvent. These relationships for a few common extraction solvents are given on Table VII. While the latent heat of vaporization is important, the total heat to vaporize a gallon of solvent is even more important. By this analysis hexane and heptane require the least heat and isopropanol the most.

TABLE VI. Isomer Comparisons in Cottonseed Extractions

Run No.	Solvent	Extraction Temp., °F.	Cooked	Oil in Meal, %	FFA, Wt. %	Refining Loss, Wt. %	Color Transmission, Refined Oil, %
A-1	Hexane	122	No	2.7	4.7	17	--
A-2	Methylpentanes	122	No	2.3	6.9	20	--
B-1	Hexane	133	No	2.6	2.2	9.6	91.1
B-2	Methylpentanes	131	No	2.4	2.6	7.5	93.5
C-1	Hexane	127	Yes	5.7	1.0	8.0	83.6
C-2	Methylpentanes	124	Yes	4.1	1.4	5.8	82.0
D-1	Hexane	132	Yes	3.2	2.2	9.8	73.0
D-2	Methylpentanes	135	Yes	2.6	1.9	7.1	86.0
E-1	Hexane	134	Yes	4.8	2.2	10.2	69.0
E-2	Methylpentanes	135	Yes	3.6	2.2	9.2	82.7

Hexane - b.p. 150-156° F., 70% n-Hexane, 19% MCP, 6% 3-MP, 5% Other C_6.
Methylpentanes - b.p. 135-147° F., 60% 2-MP, 26% 3-MP, 5% n-Hexane, 6% 2,3-DMB, 3% Other C_6.
Conditions: Modified NURDD Laboratory extractor; continuous and countercurrent; containing components of commercial unit.

TABLE VII. Physical Properties of Some Extraction Solvents

	n-Hexane	n-Heptane	Tri-chloro-ethylene	CS_2	Isopro-panol	Acetone
Boiling point, °F.	156	209	189	115	180	133
Molecular weight	86	100	131	76	60	58.08
Specific gravity, 20/4	.6594	.6838	1.4642	1.259	.785	.792
Viscosity, cps @ 30°C	.44	.45	.54	.37	2.08	.33(20° C.)
Latent heat of vaporization at b. p., BTU/lb.	144	138	102	150	288	222
BTU/gal.	790	788	1220	1520	1890	1463
Specific heat @ 60°F.	.45	.53	~.3	.24	.61	.511 (20° C.)
Surface tension, dynes/cm. @ 20°C.	18.4	19.3	29.3	33.1	21.7	23.2
Flash, COC, °F.	-15	+25	None	-22	53	+15
Inflammability limits in air						
- Lower-Vol. %	1.2	1	- -	1.0	2.0	2.5
- Upper-Vol. %	6.9	6	- -	50.0	8.0	12.8
Solubility - 25° C.						
in water, gm./100 gm.	0.0145	0.0150	0.11	0.22	∞	∞
water in solvent, gm./100 gm.	0.0130	0.0070	0.022		∞	∞
Approx. BTU's to heat 1 gal. to its b.p. and vaporize	956	1118	1536	1557	2212	1642

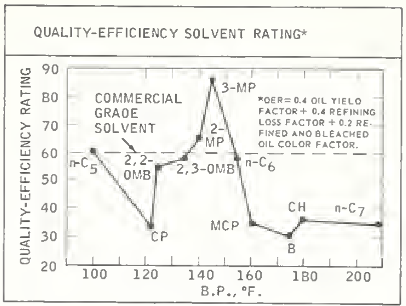

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Figure 1

Narrow boiling - Since most extraction solvents are not individual compounds, they generally boil over a range corresponding to the boiling points of the various isomers. It is desirable to have this boiling range as narrow as possible. A wide boiling range material tends to exhibit excessive solvent losses. With a high front end volatility, the solvent experiences high losses due to leaks and escape through the condenser. Solvents with a heavy back end experience losses by leaving residues in the extracted oil and meal. Additional heat or steam can usually remove these last traces, but this is wasteful of heat and can destroy the protein content of the food. In most countries using solvent extraction, solvents do not have boiling ranges greater than 10°F.

Noncorrosive - Because solvents contact the piping and other metal components of extraction equipment, it is important they be noncorrosive. Hydrocarbons are not a problem in this regard. The chlorinated and oxygenated solvents are intermediate. Carbon disulfide, of course, is very bad.

Nontoxic - The result of leaving residues of a toxic solvent in extracted meal would be disastrous. So solvents used for extraction of food materials should be nontoxic. In addition, the operation of extraction plants should insure that solvent is essentially completely removed from the meal. U. S. plants observe rigorous safeguards. Thus, the oral toxicity of extraction solvents does not seem to be a particular problem.

The toxicity of the solvent in air is important due to exposure by the operating personnel of the extraction plant. Some of the maximum allowable concentrations in air (8) are given in Table VIII. Accordingly, the safest of these solvents is acetone followed closely be hexane and heptane. Benzene has a very low MAC of 25 ppm. However, as an impurity in hexane it does not present an air toxicity problem until it reaches 5% concentration.

TABLE VIII. Toxicity

	Inhaled (MAC, ppm)
Hexane	500
Benzene	25
Carbon Disulfide	20
Trichloroethylene	100
Toluene	200
Acetone	1000
Heptane	500
Dichloroethane	50

Nonflammable - Table VII showed the flash point and flammability limits of several solvents. The hydrocarbons such as hexane are flammable and must be so treated. They are less flammable than carbon disulfide but more so than trichloroethylene. Adequate safe-guards have led to many years of safe operation with hexane. There has also developed a vast experience of safe commercial designs, and the construction code for such flammable compounds is well established. Trichloroethylene was initially selected as an extraction solvent based on its reputation for being nonflamable. The Bureau of Mines (9) has recently found that the vapors of trichloroethylene are flammable under conditions which occur in its normal use. Plant operators should be cognizant of this potential hazard.

TABLE IX. Suggested Specifications for "Food Grade" Hexane for Defatting Oleaginous Food Materials

Test	Premium Product		Average Product		ASTM Test Method
	Min.	Max.	Min.	Max.	
Distillation, °C.					
Initial boiling point	65	--	63	- -	D1078 using
Dry point		70		71	thermometer
Temp. range final 10%, °C.		2		3	39C(48/102° C)
Reaction of residue	Neutral to Methyl-Orange		Neutral to Methyl-Orange		
Composition vol. %					
Aromatics	- -	1.0	- -	3.0	D1017
Saturates	98.5	- -	96.5	- -	D1319
Bromine No., g/Br/100g	- -	1	- -	1	D1159
Specific gravity @ 15.5/15.5°C.	- -	0.685	- -	0.690	D1298
Color, Saybolt	30	- -	30	- -	D158
Sulfur, ppm by wt.	- -	25	- -	100	D1266
Corrosion, 3 hrs. @ 50°C.					
Bomb method	- -	1	- -	1	D130
Doctor	Negative		Negative		D434
Nonvolatile matter	- -	(0.0005)	- -	(0.0005)	D1353
g/100 ml.	- -	(0.0001)	- -	(0.001)	D1353
Lead (e.g. Tetraethyl Pb)					
ppm as "Pb"	- -		- -		D1368
Phosphate (e.g. Tricresyl-					LIC330.70B
phosphate) ppm as "P"	- -		- -		& D1091
Chlorine, ppm as Chlorine	- -	20	- -	20	LIC270.14

Impurity-free - Not only must the solvent itself be nontoxic but it must be free of toxic impurities, both volatile and nonvolatile. Volatile impurities such as benzene in hexane can have an effect on the hexane's air toxicity if present in great enough concentrations. Nonvolatile material in solvent hexane was the subject of an Esso Research paper presented this past year in the Journal of the American Oil Chemists' Society. (10) It showed that the hexanes studied contained only a few parts per million NVM and none of it was determined to be of a harmful polynuclear aromatic type. The purity of solvents is continually under study and faster and easier methods for determining the presence of minute impurities are under investigation.

Available and cheap - To an oilseed processor a reliable source of solvent having a uniform quality is an obvious requirement. Hopefully, this solvent will also be inexpensive since solvent losses occur due to leaks, adsorption, spills, etc. On the basis of being available and economical, hexane has competed very favorably.

Water insoluble - Live steam stripping and direct water condensing of solvents in extraction plants are common practice. Solvent is easily recovered in simple separators if its solubility in water is small. Here again the hydrocarbon solvents seem to enjoy an advantage by being relatively nonpolar and dissimilar to water and hence insoluble in it.

Based on the eighteen or so properties discussed above, the best all round performer is hexane. Because it is the solvent most used and accepted by the extraction industry, UNICEF of the United Nations has set forth some suggested specifications for "food grade hexane." These are outlined on Table IX. It should be pointed out that these are only suggestions and do not constitute rigid specifications. The tests herein cover most of the important properties. Hopefully, the world wide adoption of similar specifications for "food grade Hexane" will be forth-coming in the not too distant future.

Solvents for Extraction of Dry Materials

The solvents for extraction of wet (> 10% water) materials need the same characteristics as those used for extraction of dry materials. In addition they need to be efficient in removal of water. Thus, desirable solvents for wet materials have the properties shown in Table X. Table XI shows some of the general methods which have been used to remove water. The last of these, azeotropic distillation, is the one most pertinent to this discussion. Solely as a means of removing water, azeotropic distillation is not very efficient. However, as Table XII shows, there are other advantages. During the process of removing water, the oil in the tissue is also being extracted. So the imput heat is performing two functions simultaneously. This heat conservation is one of the major advantages for azeotropic extraction.

TABLE X. Desirable Solvent Characteristics
For Extraction of Wet Materials

1. Oil Miscible
2. Selective
3. Stable
4. Unreactive
5. Low Specific Heat
6. Low Latent Heat
7. Low Boiling
8. Narrow Boiling
9. Low Viscosity
10. Low Specific Gravity
11. Noncorrosive
12. Nontoxic
    - Inhaled
    - Ingested
13. Nonflammable
14. Impurity Free
    - Volatile
    - Nonvolatile
15. Inexpensive
16. Available
17. Water Insoluble
18. Efficient Water Remover

By using an azeotrope process, water can be removed from tissues at temperatures lower than the boiling point of water. So extractions can proceed at relatively low temperatures where protein degradation should not occur. Hence, more of the protein is biologically available. Most offensive odors and water pollution are completely eliminated. Also, the production of "fines" is much less than in a straight solvent extraction where higher temperatures are used. Generally, when extracted above 212° F., proteins become brittle and easily fragmented.

TABLE XI. Methods of Water Removal

1. Drum Drying
2. Spray Drying
3. Freeze Drying
4. Dissolution
5. Heat Transfer
6. Azeotropic Distillation

TABLE XII. Advantages For Azeotropic Extraction

- Dual Purpose
- Low Temperature
- No Fines

Some physical properties of some useful azeotropic solvents are given in Table XIII. Like solvents used in the extraction of dry materials, it is important for these solvents to possess low water solubility and a low normal boiling point. More important, however, they should have a low azeotropic boiling point and a small solvent to water overhead ratio. If this ratio is too large the heat requirements for the process become excessive. Normally the boiling point and the water overhead ratio must be compromised. At higher temperatures the water overhead becomes greater but the possibility of degrading the protein is also greater.

To date, the solvents which have been used commercially are trichloroethylene, dichloroethane, and extraction-grade heptane. In water removal capability heptane appears to have an edge. Some other hydrocarbons are listed which could be useful azeotropic solvents if they become available in large quantities at an economical cost. Because of the increasing importance of linear alkanes in the manufacture of biodegradable detergents, it is possible that more n-heptane may become available at a reasonable price. n-Heptane is a better water entrainer than the commercial grade is. Isooctane removes water equal to n-heptane and has a lower latent heat. But it is presently too expensive to be used commercially. A cheap mixture of isooctanes appears to have properties comparable to that of isooctane.

Conclusion

In conclusion, it appears that extraction solvents can be conveniently placed into two categories. One category, used for relatively dry materials, includes the low molecular weight hydrocarbons and other solvents which do not need a water removal capacity. The other, for wet materials, requires the water removing capability. In the first category the new solvent ideas appear to be the use of mixtures of solvents rather than the discovery of any new individual candidate. As far as an individual solvent is concerned, commercial hexane still seems to be the best compromise. All it lacks is the nonflammability characteristic. The small performance advantage for methylpentanes probably is not worth their premium price.

In the extraction of wet materials, dichlorethane, trichloroethylene, and heptane seem to perform satisfactorily. However, other materials may become available which could out-perform these solvents in water removal and cost less.

TABLE XIII. Physical Properties of Azeotropic Solvents

	Dichloroethane	Trichloroethylene	Perchloroethylene	Chlorobenzene	Benzene	n-Heptane	Heptanes*	Isooctane (2, 2, 4-TMP)	Mixed* Isooctanes	n-Octane
Specific gravity @ 20°C.	1.253	1.464	1.623	1.105	0.879	0.684	0.7298	0.692	0.6964	0.707
Water solubility @ 25°C., g/100g.	0.84	0.11	0.015	0.049	0.07	0.005	0.015	0.015	0.015	.002
Normal b. p., °F.	182	189	250	270	176	209	200-210	210.6	209-217	258
Latent heat @ b. p., BTU/lb.	140	103	90	140	168	137	136.5	116.7	119.5	129.5
Azeotropic b. p., °F.	160	164	190	195	156	175	176-185	176	176	193
Solvent/water, Wt.	11.3/1	13.4/1	5.3/1	2.5/1	10/1	6.7/1	8.7/1	7.7/1	7.3/1	2.9/1
Water overhead, Wt. %	8.0	6.9	15.9	28.5	9.1	12.8	10.0	11.6	12.0	25.5

*Experimental values

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DISCUSSION

V.L. Frampton: I wonder if you could offer us any comment on the freons as solvents?

J.A. Price: Well, I think they would be good things to try. Just offhand, I don't know of any that have a high enough boiling point. My guess, based on the lower members of the freon family, is that stability-wise they should be better than the nonfluorinated solvents. I can't offer you too much more on that.

PROGRESS IN BREEDING GLAND-FREE COTTONSEED

by
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The scientific name for the group of plants to which cotton belongs is Gossypium. All species and varieties are characterized by small, darkly pigmented glands, and it is appropriate that the most prominent of these pigments is called gossypol, a word derived from Gossypium. The leaves, stems, flowers, and bolls have these glands, but most importantly, these glands containing gossypol and other pigments in the seed are a nuisance to the cottonseed crushing industry since they discolor the oil, and lower the nutritive value of feed, and the toxicity of gossypol limits the use of cottonseed meal in animal rations, particularly poultry and swine.

The possibility that these glands containing gossypol could be eliminated from cottonseed rests on basic genetic investigations conducted by McMichael (1960) of the U. S. Cotton Field Station, Shafter, California, followed by supporting evidence from Miravalle and Hyer (1962) and Lee (1962). McMichael began to report on his efforts to develop strains of cotton without glands in the seed as early as 1954. By 1960 experimental strains with virtually no gossypol in the seed were available, and the inheritance of the character was published. The essential features of the genetics are shown below:

$Gl_2Gl_2\ Gl_3Gl_3$ = glanded (gossypol-containing) seed
$gl_2gl_2\ gl_3gl_3$ = glandless (gossypol-free) seed

Theoretical result of crossing a glanded with a glandless strain of cotton.

$Gl_2Gl_2\ Gl_3Gl_3$ X $gl_2gl_2\ gl_3gl_3$

F_1 hybrid, $Gl_2gl_2\ Gl_3gl_3$ - reduced glands on boll and in mature leaves; seeds normally glanded.

Theoretical results of self-pollinating F_1 hybrid, $Gl_2gl_2Gl_3gl_3$

Ratio	Genotype	Phenotype
1	$Gl_2Gl_2\ Gl_3Gl_3$)	
2	$Gl_2gl_2\ Gl_3Gl_3$)	
2	$Gl_2Gl_2\ Gl_3gl_3$)	
4	$Gl_2gl_2\ Gl_3gl_3$)	Glanded to some extent
1	$Gl_2Gl_2\ gl_3gl_3$)	
2	$Gl_2gl_2\ gl_3gl_3$)	
1	$gl_2gl_2\ Gl_3Gl_3$)	
2	$gl_2gl_2\ Gl_3gl_3$)	
1	$gl_2gl_2\ gl_3gl_3$-	Glandless seed, cotyledons, stipules, and leaves

Theoretical results of crossing F_1 hybrid with a glanded variety.

$Gl_2gl_2\ Gl_3gl_3$ X $Gl_2Gl_2\ Gl_3Gl_3$

Ratio	Genotype	Phenotype
1	$Gl_2Gl_2\ Gl_3Gl_3$	Glanded
1	$Gl_2gl_2\ Gl_3Gl_3$	Glanded
1	$Gl_2Gl_2\ Gl_3gl_3$	Glanded
1	$Gl_2gl_2\ Gl_2gl_2$ -	Reduced number of glands on bolls

The inheritance of glandless seed as outlined will be recognized by students of genetics as a classical case in which the expression of a character is controlled by two factors or genes.

The first glandless strain was derived from a cross between Acala and Hopi Moencopi, a noncultivated primitive cotton from Arizona. This original strain had glandless seed, but it also had small bolls, low lint percentage, inferior fiber properties, little disease resistance, late maturity, rank growth, and low yields. Obviously, the value of glandless seed could not overcome the undesirable features, and it was economically out of the question to attempt to grow this cotton commercially. If glandless seeds were ever to be of value to the cotton industry, it was necessary for breeders to attempt to develop glandless strains of cotton which were not inferior in any important respect to the commercial varieties of glanded cotton.

The breeding problem was not to improve the glandless (low gossypol) seed, per se, but to combine the glandless seed character with the total complex of desirable traits necessary in a successful commercial variety, or, if you prefer, to disassociate glandless seed from the undesirable features inherent in the original glandless stock.

The original glandless variety had only one desirable feature, glandless seed, while the commercial varieties represented the best combinations of all other agronomic and fiber considerations. The backcross breeding method is usually advocated for a situation where the problem is to introduce a single and simply inherited trait from an otherwise inferior line into commercial varieties. As far as breeding for glandless seed is concerned, the backcross system means that, after each backcross, plants having the two genes necessary for the production of glandless seed are recognized and crossed again to the recurrent parent. Miravalle and Hyer in California and Meyer in Mississippi have developed practical techniques for recognizing and proving the desired plants. The process is repeated, generation after generation, until the backcross progeny has the essential features of the recurrent parent; then, following self-pollination, true breeding glandless lines are recovered and tested. Theoretically six or seven backcrosses should be sufficient provided no hard-to-break linkages or deleterious pleiotypic effects are encountered.

It should be emphasized at this point that several backcross programs needed to be undertaken simultaneously, each employing different varieties as recurrent parents. The different cotton growing areas have different problems in regard to climate, soils, diseases, insects, harvesting methods, and quality considerations. No one variety is universally well suited to the whole country. So, if the whole belt were to be served in this breeding attempt, several different varieties had to be used as parents. In order that all varieties would be involved in glandless breeding, the Delta Branch Experiment Station at Stoneville, Mississippi, after a survey of work in progress engaged in an extensive program to carry ten commercial varieties at least as far as the fourth backcross. This program has now reached the final stage and has already contributed importantly to glandless pollen availability in Iguala.

After the genetics of glandless seed was understood, seed stocks were officially released to all interested public and private breeders and each year the availability of pollen from glandless plants was announced at the winter cotton breeding facility at Iguala, Mexico. The National Cottonseed Products Association was interested in glandless breeding from the outset and has supported the work with grants-in-aid at Shafter, California, and the Delta Branch Experiment Station, Stoneville, Mississippi. Public as well as private seed breeders obtained glandless seed stocks and utilized the pollen made available in Iguala. The combined efforts of the public and private cotton breeders was sufficient to involve every important cotton variety in a backcrossing program. Varieties of Coker, Empire, Stoneville, Deltapine, Rex, Auburn, Dixie King, Lankart, Gregg, Paymaster, Acala, Pima, and other types of cotton were used as backcross parents in order to introduce the genes for glandless seed into this array of varietal types. These varietial types account for well over 90 percent of the cotton acreage in the United States. It would be a mistake to leave the impression that breeding for glandless seed suddenly became the major objective of all these breeding programs. The backcross program by its very nature is designed to return to the characteristics of the recurrent parent, but would not be expected to result in much improvement other than the glandless seed feature. When glandless seed became a breeding objective, the breeders had active programs to improve glanded cotton in respect to yield, quality, disease resistance, and other characters. Breeders are reluctant to abandon or de-emphasize their main objectives and engage in a crash program for glandless seed. Private breeders were kind enough to reply to letters of inquiry of mine regarding the status of glandless breeding. The emphasis being given to glandless breeding varies, but it is fair to say it is a serious, although not primary objective, of the commercial companies. They have not been disappointed in their progress. In other words, they are not having any particular trouble handling the character genetically and they have not observed any obvious deleterious associations with glandless. Most of them have glandless strains going into preliminary performance trials.

Breeders attempting to develop glandless varieties of cotton are reluctant to forecast the time for commercial release. None of the companies replying to my letter of inquiry have prospects for release within the immediate future, and the task of breeding glandless commercial varieties is more complex than simply making six or so backcrosses to a commercial variety, recovering a true breeding glandless strain, testing it in performance trials, and making a commercial release. As stated previously, glandless breeding is a serious but not primary objective; the breeders are making progress in breeding for yield, quality, disease resistance, boll type, etc., on a glanded background. It is possible that by the time a glandless version of a current variety is developed, an improved new glanded variety may be superior. In order that glandless breeding will not always be a step behind glanded breeding, two approaches are now being used. First, the backcross program is not confined to current commercial varieties. The most promising new strains are also used as backcross parents. Second, the backcross method is no longer necessarily the logical breeding method. Glandless strains of most agricultural varieties are available very nearly as good agronomically as their glanded counterparts. No one uses the old original glandless strain with all its agronomic faults as a parent any more. They choose instead the most upgraded glandless strains on the varietal background of interest. Crosses may be made and selection practiced immediately. In other words, efforts to improve cotton in a number of respects goes on as before except on a glandless background. Varietal releases in the future from this approach would be made for the same good reasons that varieties have traditionally been released and the glandless seed feature would be an extra dividend or bonus.

Glandless seed is a completely new goal for cotton breeders. They have never been challenged before by a useful, simply inherited character not influencing yield or lint quality. Perhaps this is the reason the approach is one of caution rather than crash programs for glandless seed. They want to make sure that no deleterious associations or bad side effects of the glandless genes exist. One of the most advanced glandless breeding programs is that of the Shafter station in California. Glandless Acala types extracted from the fourth backcross perform about as well as Acala 4-42 in yield, quality, and other agronomic features. Some of the glandless strains tend to be later in maturity and ranker in growth. The breeders have reason to believe that these traits are not byproducts of the glandless nature of the strains, and that no serious obstacle is in the way of successful glandless cottons. Another advanced glandless breeding program is that of the Rex variety in Arkansas. Mr. Moosberg had a glandless version of Rex Smoothleaf extracted from the sixth backcross in yield trials in 1963. If the results over the next season show that glandless Rex is as good in every respect as Rex Smoothleaf, obviously serious consideration would be given to making an official release of the new glandless variety.

Reports have been received from entomologists that glandless cotton is more susceptible to insect attack than glanded cotton.

The theory is that gossypol or some unidentified substance in the pigment glands imparts some degree of insect resistance to glanded cotton to the extent that certain insects prefer glandless to glanded strains. These reports are offset to some extent by the experience of breeders who have produced several acres of glandless without unusual insect problems. The whole question of the possible influence of glandless on the insect problem is most complex. Breeders and entomologists are both aware of this problem, and cooperative research in this area is contemplated.

Another consideration is the incentive for developing glandless cottons. In our society, commercial endeavors are motivated primarily by economic considerations and profits. In the case of glandless seed, the breeder, the seedsman, the farmer, the oil mill, and the oil mill's customers form an interrelated group, and the success of the whole venture depends upon the success of each element in the order named. The breeder must develop glandless cottons as good as or better than glanded ones in all other respects. The seedsman must then dare to put the cotton on the market. The farmer must decide to grow the glandless cotton and in volumes large enough to supply oil mills, and the oil mills must crush the seed. Finally the customers of the oil mills must become familiar with the products of glandless oil and meals. All reports I have heard from oil chemists and feed experts indicate that glandless cottonseed would be a tremendous benefit to the cottonseed crushing industry. My only point in the previous speculation is that the price of glandless seed products would be established in the market place and passed backward to the oil mill, the farmer, the seedsman, the breeder whereas the original development must come in the reverse order. The first firm to release a glandless variety may have to do so with some faith and risk until the seed prices have a chance to seek their own level. The main incentive now perhaps is the realization among seedmen that if glandless varieties become a reality, glanded seed might rather suddenly become obsolete. No responsible breeder wants to risk his competition getting to far ahead in this respect.

Summary

1. Experimental strains of cotton have been developed without the darkly pigmented glands in the plants, and these glandless strains have virtually no gossypol in the seed.

2. The inheritance of the gland-free condition has been published and seed stocks and pollen made available to public and private breeders.

3. Since the original glandless strain is agronomically inferior to commercial varieties of cotton the breeding problem is to develop glandless cottons which are not inferior in any important respect to commercial varieties of glanded cotton.

4. The total effort of public and private breeders involves varieties presently accounting for over 90 percent of the cotton acreage.

5. Glandless breeding is a serious although not primary breeding objective of most commercial cotton breeding firms.

6. Breeders are beginning to enter glandless strains in their performance trials; they are reluctant to forecast a date for the release of glandless varieties.

7. Progress has been encouraging, and no insurmountable obstacle to the eventual development of glandless cotton is known. The most serious potential problem is that of increased insect susceptibility.

8. The advent of glandless varieties rests on the biological success of the breeder becoming intergraded into the economic system involving the seedsman, farmer, oil mill, and ultimately the consumer of cottonseed products.

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DISCUSSION

G. A. Harper: I wonder if you would comment on the possible availability of this glandless material to breeders and through them to growers in areas outside the United States.

C. F. Lewis: The Department of Agriculture officially released glandless type cotton some years ago, so we consider that this type of cotton is free for distribution. Obviously, a commercial company may not want to give away their pet material, but you can get a very good version of glandless cottons certainly from the Stoneville program, the California program; and it has already been placed abroad, if this is what you mean. I know the Brazilians have it, the Argentines, and the Mexicans. I am sure the British Empire Cotton Growing Corporation has received seeds of this, as have the French cotton industry in Africa. There is no prohibition against any private breeder in the United States or abroad, as far as I know, with the exception of some of the iron curtain countries. I should think that the cottons coming out of the Stoneville program would be most appropriate. All you need to get started, if you have a breeding program, is anything that has these two genes in it and you can go on from there.

Max Milner: I wonder if we could ask scientists from other countries who are probably more directly concerned with the food use of cottonseed, whether they would care to comment on the progress of such studies in their countries. I would like to suggest, for example, Dr. Bacigalupo. Do you have any comments to make on progress in Peru?

Antonio Bacigalupo: Yes, in Peru, our geneticists are working on improving the native species of cotton that we have, gossypium barbadense and hirsutum, with these genes of glandless characteristics. Already some progress has been made and tests are being run on the F_1 generation. We still don't have enough results to report the final outcome. I think in this program we are following the first line of development that you mentioned, Dr. Lewis, that is backcrossing to the native varieties the characteristics of the glandless. The world market requires certain specifications as far as the fiber is concerned and we want, of course, to retain a world market for our own Peruvian cotton. Now, for internal consumption, most of the varieties that we grow are very high in gossypol and it would be a very good combination to have a Tangüis variety with the characteristics of the fiber without the glands that contain large quantities of gossypol. In this program, we are making a survey of the characteristics of different strains and the species of cotton that we have in Peru and we are trying to pick those which could be improved by the glandless characteristics. Of course, the nutritionists are following this closely since our main objective is to produce a high quality protein from this seed. I would like to ask Dr. Lewis if any studies have been made in connection with the nutritive qualities of the glandless varieties, especially in connection with the biological value, and also about the color of the meal produced from the glandless seeds. Is it yellow or is it a whitish meal?

C. F. Lewis: I think this is the subject of our next speaker, to explain the seed trials and the nutritive values and the colorless properties of gland oils and meals, so he will be better informed than I am on this.

B. H. Wojcik: Anyone else from abroad that would like to say something on the subject?

C. D. Kapsiotis: Mr. Chairman, I would like to give some information in connection with developments in our organization. Our Plant Production and Protection Division has an interest in the development of glandless varieties of cotton. I know of one example in Teheran, Iran, where in the Cotton Research Station they are continuing breeding work on glandless cottonseed. This work was initiated by an FAO cotton breeding expert with seed brought from Chad and with which the French Government had started a few years ago. But, as I understand it, this is not a large-scale operation and it is not spectacular compared to the work on glandless seeds in this country. We do plan to request our cotton breeding and production experts to focus attention on the glandless cottonseed.

PROPERTIES OF GLANDLESS COTTONSEED

by
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For many years, and very intensely since 1950, the properties of glanded cottonseed oil and meals have been studied. Much progress has been made but the last few years have not been as productive of results as was desired because of the presence of gossypol in the seed and its presence in the resulting oil or meal. The original objective of these nutritional studies was to produce cottonseed meals that could be used in unrestricted amounts in swine or poultry rations. This has almost been realized but the feeding of cottonseed meals to swine and poultry in unrestricted amounts is still hazardous.

With the development of glandless strains of cottonseed which were free of gossypol, it was hoped that the original objective could be realized. It seems appropriate that the properties of these new varieties should be studied. This paper will confine itself with the consideration of the lipid and protein fractions.

The lipid fraction of glandless cottonseed has been studied by Thaung, Gros, and Feuge (7). The conventional glanded varieties contain gossypol which, if present in the oil, contribute to a color problem. Certain characteristics of the lipid fractions of the two types of cottonseed are presented in Table I. It will be noted that the free fatty acids are low and not markedly different. The saponification and iodine values fall in the range of values to be expected. This was also true of the unsaponifiable material, which was higher than normal for the glanded and lower for the glandless. Glycerides of saturated acids were present in about the same amounts. The total oleic acid glycerides were slightly higher in the glandless but no significance was attached to this observation. Those of linoleic acid were present in essentially the same amounts. The fatty acid composition of the two oils was quite similar as regards palmitic, oleic, linoleic, and stearic acids. The two oils contained similar amounts of diene and triene conjugation.

The oils of both cottonseed reacted positive to the Halphen test and to about the same extent. This is believed to be due to the presence of a fatty acid containing a propene ring. This observation is further enhanced by Frampton (1) who indicates that hexane extracted oil from cooked glandless cottonseed fed to laying hens resulted in eggs in which pink whites occurred in 9 out of 17 eggs and enlarged yolks were observed in 10 of 17 eggs. If the hexane-acetone-water mixed solvent system was used to extract the oil, all of the eggs contained pink whites at the end of 3 months storage.

The only major difference between the lipid fractions of glandless and glanded cottonseed is the absence of gossypol in the glandless. No typical egg yolk discoloration of gossypol is observed when glandless cottonseed oil is fed to laying hens.

The initial consideration concerning the protein fractions of glandless cottonseed is the amino acid template. The content of certain amino acids of both glandless and glanded cottonseed is presented in Table II. The values were determined by the conventional chromatographic procedure. The values for glandless cottonseed proteins is from the paper by Martinez and Frampton (5) and that of the glanded is from Miner (6). It will be observed that the contents of the two types of cottonseed are essentially the same.

Some two years ago a limited quantity of glandless cottonseed became available for use in testing the nutritional value of the resulting cottonseed meal (2). The purpose of this

TABLE I
CHARACTERISTICS OF OILS FROM GLANDED AND GLANDLESS COTTONSEED

Characteristic	Glanded	Glandless
Free fatty acids, as oleic	0. 88	0. 21
Iodine value, Wijs	106. 6	108. 0
Saponification value	192. 8	192. 6
Unsaponifiable matter, %	1. 7	0. 5
Glycerides of:		
Saturated acids, %	29. 7	28. 5
Total oleic acid, %	19. 4	22. 6
Linoleic acid, %	49. 2	48. 4
Fatty acid composition:		
Palmitic	27. 2	26. 5
Oleic	20. 1	18. 8
Linoleic	43. 8	46. 0
Stearic	3. 1	2. 9
Conjugation:		
Diene, %	0. 89	1. 01
Triene, %	0. 01	0. 01

TABLE II
AMINO ACID CONTENT OF COTTONSEED PROTEINS

Amino Acid	Glandless	Glanded
Lysine	4. 3	3. 4-4. 3
Methionine	1. 5	1. 6
Threonine	3. 4	3. 5
Proline	3. 6	3. 8
Valine	5. 0	4. 4
Isoleucine	3. 2	3. 1
Leucine	5. 8	5. 7
Histidine	2. 7	2. 5
Phenylalanine	5. 8	5. 5

study was twofold. One objective was to determine the nutritional value of cottonseed meal prepared in the complete absence of gossypol. The second objective was to test the hypothesis that gossypol was binding with the protein or lysine of the protein. The results of this trial are presented in Table III. A study of these data yields two observations:

1. The evidence indicates that gossypol is reacting with the protein or amino acids to reduce the nutritional value.

2. Hexane extraction of glandless cottonseed produces meals that have low nutritional value which would not respond to lysine supplementation. Mild cooking of this meal produces a decided improvement in nutritional value.

TABLE III
PROTEIN EFFICIENCIES OF GLANDLESS COTTONSEED

Seed	Solvent	No Additive		0.3% Lysine	
		Uncooked	Cooked	Uncooked	Cooked
Glandless	Hexane	1.65 1/	2.22	1.66	2.28
	Gossypol added		2.06		2.36
Glanded	Mixed solvent	2.27		2.41	
Commercial	Cottonseed Meal	1.88		2.16	

1/ Gain per gram of protein consumed.

A further study with glandless cottonseed involved the use of two solvent systems and two heat treatments. The mixed solvent system developed by Mann et al (4) and hexane were the two systems used. The meals were prepared without the use of heat and with mild cooking. These results are presented in Table IV. The meals prepared by hexane extraction responded the same as the previous trial. Mixed solvent extraction produced meals of high nutritional value that were not improved by mild cooking.

TABLE IV
THE EFFECT OF HEAT AND SOLVENT SYSTEM ON PROTEIN EFFICIENCIES
FROM GLANDLESS AND GLANDED COTTONSEED MEAL

Seed	Solvent	No Additive		0.3% Lysine	
		Uncooked	Cooked	Uncooked	Cooked
Glandless	Hexane	2.02	2.51	2.20	2.72
	Mixed Solvent	2.94	2.80	3.17	3.06
Glanded	Hexane	1.11	1.79	1.10	2.30
	Mixed Solvent	2.52	2.13	3.00	2.84
Commercial Glanded Cottonseed Meal		1.92		2.52	

These studies suggested to Johnston (3) the possibility of a factor or factors that are present in glandless cottonseed and prevent the maximum utilization of the protein. This factor would be insoluble in hexane, soluble in the mixed solvent system, and labile to heat. Preparation of extracts and residues in an attempt to characterize this factor have met with little success (Table V).

This difficulty along with the observation that the rations containing the hexane extracted uncooked meals gummed up on the beaks of the chicks led Johnston (3) to postulate that the cooking and mixed solvent extraction were producing a physical change in the meal that would explain the nutritional response. To measure this he ran infrared absorption curves on the various preparations. The absorption curves of the hexane extracted uncooked meals showed absorption at 3.25 microns and at 6.00 microns. The absorption at these two places decreased or disappeared as the meals were heated. The extraction with the mixed solvent system produced similar changes. This was interpreted by Johnston as indicating that the unheated meal is in a super-folded beta form. He postulates that the heating of the hexane extracted uncooked meals reduces the intermolecular hydrogen bonding and makes the proteins more available nutritionally. The polar nature of the mixed solvent system may be responsible for similar changes. These observations are of a preliminary nature but they do offer a hypothesis which adequately explains the nutritional changes observed.

Finally the results of a practical feeding trial are presented in Table VI. In this trial glandless cottonseed meals were prepared simulating the several commercial production processes. It will be noted that the glandless cottonseed meals in this trial were fully equal to soybean meal in nutritional value. Also the mixed solvent extraction produced meals that were no better than the simulated commercial meals. If it can be assumed that these results are indicative of what can be done commercially with glandless cottonseed, then the original objective of the glanded cottonseed research will be realized with this type of cottonseed.

TABLE V
RESULTS OF FEEDING TESTS WITH FRACTIONS FROM GLANDLESS COTTONSEED

Extraction Procedure	Extract	Residue
H.A.W. Extraction	3.42 1/	2.48 1/
H.A.W. Extraction Plus Heat	3.43	2.52
Hexane Extraction	3.37	2.60
Hexane Extraction Plus Heat	3.22	2.56
Corn Oil	3.37	
Corn Oil Plus Heat	2.42	

1/ Gain per unit of protein consumed.

TABLE VI
RESULTS OF EIGHT WEEK FEEDING TRIALS WITH GLANDLESS AND GLANDED COTTONSEED MEALS

	Protein* Efficiency	Gain per Chick day (gms.)	Feed per Chick day (gms.)
Glandless Solvent Plus Heat	2.27	22.95	48.19
Glanded Solvent Plus Heat	1.98	15.74	37.92
Glandless Screwpress	2.29	22.15	46.09
Glanded Screwpress	2.03	14.07	33.07
CSMA	1.96	15.13	36.74
CSMB	2.01	16.35	38.92
CSMC	1.85	15.13	38.96
SBOM	2.31	23.76	49.55

*Gain/gm. protein consumed.

By way of summary, one might suggest the following:

1. Cottonseed meals can be produced from glandless cottonseed that are fully equal in feeding value to soybean meal.

2. Because of the absence of gossypol the color problem of the crude oil is reduced and the meals can be used in unrestricted amounts in the feeding of poultry and possibly swine.

3. The absence of gossypol reduces the need for complicated solvent systems for the removal of the oil. It is doubtful that the mixed solvent system will be necessary for the production of high quality meals.

4. Glandless cottonseed contain fatty acids containing a propene ring which will cause pink white discoloration of the eggs of hens fed the oils. It is felt that meals can be produced which are low enough in oil so that this will not be a problem in the use of the meals.

5. Glandless cottonseed appear to be very similar to glanded cottonseed as regards the oil and protein components with the exception of the absence of gossypol in the glandless.

6. It is suggested that the characteristic changes necessary for the production of high nutritional quality meals can be measured by infrared absorption studies of the meals.

7. Feeding chicks and hens the oils and meals from glandless cottonseed has resulted in no deleterious effects from a physiological and anatomical standpoint.

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WHAT NEEDS TO BE DONE

by
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Some of you have remarked that you would like to have time to discuss subjects that were mentioned earlier. Perhaps the best way to do this would be for me to try to summarize my understanding of some of the things that have been said and then give you a chance to argue the point. I would like to discuss four subjects:

(1) Where do we stand now; (2) what new approaches are in the offing; (3) a look at the future; and (4) the role of fundamental research.

First, is there a process now, that is, is there a commercial process for the production of cottonseed flour suitable for human consumption? In my opinion, the answer is yes. I felt that way some time ago. At the last meeting in 1960, the late Fincher described the process for producing cottonseed flour for human consumption. My feeling has been strengthened at this meeting by Bressani, who showed in a brilliant way some of the progress that he has made in making a cottonseed flour in commercial process. I have also had private information from Mr. E. Marshack, who spent a considerable amount of time recently in designing such a plant. What would be the characteristics of such a process? It seems from what we have heard that this process would be a prepress-solvent extraction process, that this would involve proper cooking, proper control of the hull content in the pressing operation. It would have a low oil content in the concentrate, a low gossypol content, and a high epsilon amino lysine. It would have to be a cheap process, and it would be since it would be very little different from the ordinary commercial processes for making cottonseed flour.

Much has been made of the fact that cottonseed protein concentrates made in such a way and cooked later in water, alone or in the presence of calcium or iron, there is further reduction in the gossypol content. I think that is fine, but I don't think one ought to depend on this as to whether or not that is a good process. In my opinion the process exists even if the concentrate is not cooked later in water. And in that vein, I would hope that Dr. Bacigalupo, who gave an excellent description of his process, might find, perhaps as a result of the discussions here, it unnecessary to have a second cooking because this adds to the cost of his final product.

What are the weaknesses of this process? The weaknesses are that at the present time, a seed of low gossypol content is required. You have to select seeds of low gossypol content; and if only seeds of high gossypol content are available, it is rather difficult to make a product of the right properties.

The measure of gossypol itself is suspect. This was brought out by Frampton when he showed lack of correlation between gossypol content and some of the properties that he was measuring. It was brought out by Lyman when he showed that there were certain gossypol derivatives more toxic than others. And it was shown by Bressani when he was unable to obtain balances when he fed animals gossypol and tried to balance the intake and output of gossypol. I think there is a real need for further research on a biological method for measuring that component of the gossypol which is physiologically active.

Another problem with meals regardless of how they are made is the possible presence of mycotoxins. I don't consider this a dangerous or a fatal situation. I consider it something that has to be watched. In the last analysis it is an opportunity for food processors to control better the availability of food that might have a long range deleterious effect. I do think that from here on, all manufacturers of products for human and animal consumption must keep in mind this problem of aflatoxin and related mycotoxins. Certainly no one ought to try to produce a product for human food from seeds which have been damaged in storage or which have had a bad cultural history.

Now, the next point. If we do have a process which is cheap and which, despite problems, can be used in various parts of the world, why should we worry about a new process? The answer is that it would be better if you could get rid of all the gossypol. Even though the relative importance of this variable is not clear, it is still toxic and it would be better if it were removed altogether. Moreover, processors are now limited to seed of low gossypol content. If there were a process that removed the gossypol, then that limitation would be removed.

Dr. Bruins pointed out difficulties with the color of the products due in part to gossypol content. A process reducing that color would be advantageous. What do we have in that connection? Two processes were presented here at the meeting. In both instances, there is no question that the protein concentrates so produced are superior to any other cottonseed meal. Dr. Watts pointed that out very clearly for the hexane-acetone-water meal. There was evidence presented by Dr. Vaccarino of the same property in his acetone extracted meal. Both exponents claim an economically sound process for protein and oil. If either of the people corner you, and try to sell you on the idea of building a plant, it may be entirely correct. I certainly think industry ought to take a good look at these processes, and I was glad to hear Dr. Vaccarino say yesterday that they were in the process of building a plant.

I do think that there is a major theoretical difficulty in these processes that was brought out by Dr. Price yesterday in what we might call the paradox of selectivity. As you will remember, Dr. Price said if you have a solvent that is very good for oil, then you have problems of refining, you have a higher refining loss. And if you have a solvent that is a poorer solvent for oil, you have less problems of refining. You will remember from his tables that the reason for commercial hexane being the solvent of choice is that even though it is not as good a solvent as some of the others, it generates fewer problems of refining.

As Dr. Wilcke pointed out, the economic value of cottonseed has equal benefit from its oil and protein components. At the present time, with the present method of processing, the oil is good, the refining is easy, and the person who is trying to sell the protein has the gossypol problem.

With the new processes that are being proposed, the problems are now placed on the oil refiner, and I think he is no more willing to accept them than the person who has to sell the protein. I would like to suggest that perhaps these two groups should be more modest in their objectives; perhaps a two-stage approach might be one which has a better chance of initial success instead of trying to revamp completely the picture of oilseed processing. Since we know that the new protein concentrates will not be in great demand initially why not just take a small proportion of the production and try to do something with it? I was rather excited about the fact that Dr. Vaccarino started with a press cake and then extracted it with acetone. I am wondering why he quit, because that makes a lot of sense; you don't have the problem of the oil refining because most of the oil is already taken out.

And I wonder whether the hexane-acetone-water people might not take the marc from the standard hexane plant which already has one of their components, hexane, in it and go ahead and take maybe ten percent of the production and put it through the rest of their process and produce a protein that is very good. That might be a little more expensive, but would justify the expense by the additional markets for humans. In so doing they would not be doing anything really new because the soybean people are doing it now.

There is an interesting development in the soybean industry, and that is production of a protein concentrate of 70 percent protein, where they do just exactly that. A 50 percent solvent extracted soybean protein concentrate is extracted further with some polar solvent to get a 70 percent protein concentrate. There are three companies making it in the United States. There is a company in Japan, I am told, that does it with methyl alcohol. There is a company in Israel that will be on stream shortly making a 70 percent protein concentrate from soybean. Why can't the cottonseed people do the same?

Now, one might very well ask in view of the last two papers why should we worry about hexane-acetone-water or about acetone in view of the coming development of a gland-free seed? There are several answers. One, it obviously is going to take time, and what are you going to say to the people who need it right now? Two, I don't think that there really is a competition. Dr. Watts showed, for example, that hexane-acetone-water extraction of glandless seeds gave a product that was better than just plain hexane extraction. Furthermore, if you were going to make a higher protein concentrate anyhow, this would be an added advantage. You could start with a glandless seed and improve it by removing the soluble carbohydrates.

Are there any new approaches? I think one was hinted at by Price when he talked about two kinds of solvents. He talked about solvents that were used in a dry process, the conventional process, and then he talked about solvents that were used in a wet process. What would happen--I am speculating --if someone were to grind cottonseed with water and put in that water a little calcium, because Bressani and others have shown that calcium has a way of binding gossypol, and then continue the process with a solvent that would remove the oil from the water. The Russians had a process many years ago called the Skipin process which depended on adding a lot of water to cottonseed. Perhaps there might be other approaches to direct processing of cottonseed for humans. Some ideas on that have been mentioned to me by Layton Allen.

Now, let's take a long look. We are just at the point of crawling. We have 50 percent cotton concentrates made by conventional means that are good. We can see in the offing 60 percent and 70 percent concentrates that will be much better. Can we look a little further? I would like you to recall the paper given by Harold Wilcke. The soybean industry has passed the point of worrying about having products equivalent to animal proteins nutritionally; they are now thinking of other things, like structure and interest and chewiness. That is why he was showing fibrous materials. It is too soon perhaps for the cottonseed people to worry about this point, but maybe when we have the next meeting, five to ten years from now, we ought to worry about it a little bit.

One comment about cottonseed versus soybeans -- some people have said that the main reason for using cottonseed is that there are no soybeans in those areas. I suppose some people have the feeling that if it were possible to plant soybeans all over the world, then we could forget about cottonseed and peanuts and other things. We can all eat steak, but we don't eat steak every day. Sometimes we eat fish and sometimes we eat duck. It would be a monotonous world if all we could eat were soybeans. I think there is room for other products. It has been shown that mixtures of cottonseed and soybean protein concentrates are superior to either one alone. So don't short-change cottonseed, even in places where soybeans can be grown.

I would like to talk just for a moment on basic research. There is certainly a necessity for some of these practical problems to be solved, but I have a prejudice for basic research; I admit it. And I would like to point out some of the advantages of basic research. For example, many of the major problems of cottonseed and other foods is their minor constituents, and they are very difficult to identify and concentrate. Sometimes you don't even know where to look.

The way to handle a minor constituent is to make it a major constituent. The way that basic research has handled minor constituents has been to make them major constituents. If you remember some of the pictures that Yatsu showed, there were some mitochondria, but just a few. One can, by proper grinding of tissue and centrifuging, obtain a concentrate of mitochondria, and then you can study them. If you want to study ribosomes, which are the major site of nucleic acids, and nucleic acids are interesting to food people, you can fractionate seeds to obtain these minor constituents in high concentration. Everyone talks about lecithin and phosphatides and their effect on the oil and their effect on everything else; they are minor constituents. But one can isolate membranes.

Much basic research is going on now. But there is need for more. More work like the work of Martinez, where she showed the variability in the amino acid composition of the proteins, needs to be done. My own laboratory got started on cottonseed by a maneuver of the National Cottonseed Products Association. They awarded us a fellowship for basic research, and so we got Dr. Yatsu, and he started working on cottonseed. Then we hired Yatsu away from them, but he is still working on cottonseed. And we got a second fellow from Davis who is working on cottonseed. Yatsu has an assistant working on cottonseed, and so they got us very deep into this whole picture.

I would like to comment on the progress that already has been made. We have had a number of meetings on cottonseed. I can remember when it was difficult to prove that cottonseed protein concentrates varied depending upon processing conditions. Now it is a well accepted fact.

I can remember when we had no method for measuring protein quality. At this conference all of the papers clearly mentioned epsilon amino lysine as a measure of protein quality. It is accepted; it is easy to do; we now have a measure.

I can remember when we dared hope that some meals would be suitable for monogastric animals, let alone humans. This matter is now solved.

I remember when we hoped that people would come up with new processes. We have two now that we are evaluating, and we can speculate on a few more. At the last meeting in 1960, North Americans talked about processing and Latin Americans listened. At this meeting we had two excellent papers on cottonseed processing given by Latin Americans. They are not listening now. They are actually adding to our knowledge of the processing of cottonseed. This is a very healthy development.

Now, at cottonseed meetings, people are also talking about soybeans and peanuts. This is a very fine development. We have now emerged from the commodity parochialism that has limited the thinking on our oilseed problems. And now we have a physical as well as a mental picture of the inside of a cottonseed to guide us. For example, the pictures on cottonseed ultrastructure shown by Yatsu.

I would like to conclude with a paragraph taken from my book, from a chapter written by Tim Anson. He was writing about isolates from soybeans, but I think that one could take a much more general view of what he had to say, and this is what I am quoting:

> "Given nature's supply of cheap plant protein of high protein nutritional value, the technologist and the factory can do many of the manufacturing jobs that have been done at high cost only by animals.
>
> The technical possibilities of bypassing the animal have been realized so recently, however, that no one can tell to what extent and in what myriad forms they will be put into practice or how long it will take for a vast new industry to be established.
>
> My own opinion is that just as the conversion of oilseeds to oilseed meals through processing created a revolution

in animal feeding, so the further processing of oilseed meals into isolated protein, and I would say into many different kinds of protein concentrates and into complex foods containing protein concentrates, will create a second revolution. The millions of tons of oilseeds throughout the world, the basic complex raw materials which neither man nor beast can synthesize cheaply are readily available as a starting point for the new technology."[1]

DISCUSSION

B. H. Wojcik: Thank you, Dr. Altschul for an excellent summary of the meeting. Are there any questions, any discussion on the summary presented?

V. L. Frampton: There were some suggestions offered by Dr. Altschul that I think we might look at a little more closely. The suggestion was offered that perhaps we might extract prepressed meal with solvents or acetone in the preparation of meals of high quality. There are two difficulties in this. One is that under the conditions of preparing prepressed meal, you have already destroyed and bound some of the lysine. It is doubtful that this can be remedied completely on extraction with acetone, although it can be remedied in part by reversing the reaction between gossypol and lysine, or other seed constituents which may combine with lysine, by a process such as vigorous treatment with an amine. A second difficulty is that under these conditions of preparing a prepress material, you have already bound a part of gossypol, and so there is damage to the quality of the meal that probably would be difficult to reverse.

As to the question of cooking in the presence of salts of various types, this was initiated at the Southern Laboratory by Dr. Thurber and consisted of wet cooking of cottonseed meats in the presence of a base. Some of the research was with sodium carbonate and was the forerunner of the procedure that is now being used very successfully by Ranchers out in California.

Then, there is the question of mixtures of cottonseed meal and soybean meal. The statement was made that these are always superior to either meal used alone. I would suggest that the statement be amended to say that mixtures of cottonseed and soybean meals are frequently but not always better than either cottonseed or soybean meal used alone. I think that literature would not support the suggestion that the mixture is always superior.

Carmelo Vaccarino: I have a few words to say, Mr. Chairman, and I beg Dr. Altschul's indulgence in my interpretation of his talk. He suggested that we could be a bit more modest in our objectives. I am of the same opinion because I believe that modesty is the first quality of intelligent people.

Dr. Altschul discussed solvent characteristics. He told us that the solvents to be selected should relate to two objectives; first, to obtain good oil with high yields, and second to obtain a meal of good quality. Now the question is, which of the solvents possessing one of these properties is the best for obtaining the second. This is a big problem.

I would underline that the problem of extracting gossypol is not a problem, if it is taken alone. It is not difficult to extract gossypol, there are so many solvents, acetone, butanone, dioxane and alcohol that can extract gossypol; the difficulty is in the technological problem of obtaining high yields of refined oil. I should explain that at the beginning of our work our chief problem was not to obtain a better meal, but to obtain a higher yield of refined oil. You can understand the importance of this problem for us if you consider that the price of oilseeds in Italy is double that in the United States. Later we considered the advantages of getting a better meal, but for us it was of secondary importance.

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[1] Processed Plant Protein Foodstuffs, Academic Press, 1958

To obtain higher yields of refined oil with our process we separate the soap stocks in solution, not as a soft paste. We find by separating two liquids it is easier not to lose oil. I thank you.

Max Milner: I was particularly interested, Mr. Chairman, with that part of Dr. Altschul's comments having to do with parochialism. I think he is absolutely right that in future progress in this field we ought to recognize clearly the virtues and weaknesses of all of the various products and resources that are available from oilseeds. However, I am extremely unhappy that Dr. Altschul introduced another form of parochialism into his comments when he pointed out that some people seem to have the idea that it is an either-or proposition, cottonseed versus soybean. I think he would admit that it is going to be a long time before we grow cottonseed for the protein component alone. I think the major consideration of fibers will still dominate cottonseed production, and that anyone in any reasonable frame of mind would admit that all of these resources are going to have to be used, and that it is not an either-or proposition.

A. B. Watts: Two very brief comments, one, color. These meals were for all practical purposes, white, no characteristic yellow color of cottonseed at all. The mixed solvent and hexane extracted were both very, very light in color.

The other is a word of caution. I have gone through nitrogen solubility and a number of other chemical definitions of protein quality. There are many cases in our own laboratory where epsilon amino does not predict protein quality. There is a difference between the content in the protein and an amount the animal can use. As we experienced with nitrogen solubility, if you stay within the standard set of conditions, nitrogen solubility had good predictability. The very same thing is true with epsilon amino. We have got correlations as high as 0. 97 but admittedly under a narrow range of conditions, and we have also completely failed. I am giving you this word of caution because as a nutritionist I am not yet ready to completely buy epsilon amino as a predictive value for protein quality.

G. B. Cochran: I am happy to learn after 25 years of producing cottonseed flour that Dr. Altschul thinks we have finally developed a process for it. All joking aside, I think one point about cottonseed flour has been hit on lightly but not emphasized enough. We have been selling cottonseed flour of an edible grade, with low gossypol content, for 25 years. But the biggest detriment to sales for nutritional purposes or for any purpose in the United States is the color which it imparts to foods. This is one problem that we have not been able to overcome. This is one of the problems they mentioned having in Colombia, I believe, they cannot use it for a supplement in white foods. I think we look at the nutritional attributes of cottonseed flour and forget the commercial realities; people first want to start with a food they are familiar with and then use a protein for its extra added value. If the protein supplement detracts from the visible appeal then something else will be used.

There is another point I would like to mention. Traders have cottonseed flour available in very consistant quality; I think UNICEF can testify to the uniformity of our products. It is available 12 months of the year in almost any quantity that anyone desires. Quite frankly we are looking for someone who can use more than we can make. If anyone of you needs a quantity for an acceptance test or anything of this nature, we will be very happy to cooperate with you.

B. H. Wojcik: Mr. Cochran, this is a question for you. Are we paying enough attention to the metal salts that end up in the meal? What I am trying to get at, is the discoloration of the meal due to organic coloring or coloring due to metal salts?

G. B. Cochran: We think most of the coloring is due to gossypol, and this problem will be corrected with the glandless cottonseed.

M. L. Anson: I should like to come to the defense of Altschul's remark about lysine. Neither Altschul nor anyone else believes that available lysine is a complete measure of protein quality. The point is that having a chemical test for an important aspect of protein quality is a very useful achievement. If the available lysine is down the protein quality is down too, and one does not need any chicken tests to confirm this result.

To me, the main value of the lysine and other chemical tests is to detect small amounts of processing damage and thus to serve as a guide to the development and control of superior processing. The quantitative measurement and interpretation of considerable damage is of little practical interest.

C. F. Lewis: I would like to say that I have here in my briefcase the excerpt from the annual report from California which contains details of performance of Acala glandless strains. This is too much to try to put on a slide, but anybody that is particularly interested in this much detail is welcome to look at these papers privately.

H. W. Bruins: I was delighted to hear the papers this morning on the glandless meal, both from Dr. Lewis and Dr. Watts, indicating how far along we are in this whole procedure. But I wonder if we nutritionists here have not been talking a little bit too much to ourselves; what kind of economic modification do we really have to offer the cotton grower in the way of encouraging him to plant glandless seed? If we can say at the present time that all we have to offer is that it is just as good as his present varieties, then obviously he is going to have to be offered some kind of economic premium for the seed which is produced as a result. I am wondering what kind, in dollar terms of economic incentive, is going to have to be offered to this grower in order to encourage him to plant it; and in turn, what kind of incentive of our premium can the cottonseed industry afford to pay for a glandless seed.

B. H. Wojcik: Would anyone like to answer that question? Maybe Garlon Harper can do some work here.

G. A. Harper: Dr. Bruins, your question is a pertinent and justifiable one which deserves a better answer than anyone can offer because a market price is the result of the interaction of many factors which are constantly changing in their magnitude and in their relationship to each other. One can make a calculation when given a specific set of conditions, but one can be certain that these conditions will not remain static and that the market values he determines will change. The National Cottonseed Products Association conducted a comprehensive market study to obtain the best possible evaluation of the economic significance of glandless cottonseed to the grower. It definitely demonstrated that glandlessness would contribute significantly to the economic value of the cottonseed crop, but it confirmed our earlier conviction that one cannot validly assign a specific dollar increase in the price of cottonseed when glandless seed are produced.

We can demonstrate that glandless seed will improve the competitive position of both cottonseed meal and cottonseed protein. This means that under most conditions the price mills obtain for these products will, at that time, be greater than it would obtain for the same products produced from glanded seed. The buying of cottonseed is a highly competitive process and it is inconceivable that the pressure of that competition will not automatically force this advantage back to the grower through the ginner who is the first purchaser of most cottonseed.

W. T. Coleman: I am Bill Coleman from Paymaster Division of Anderson, Clayton. I am also on the Seed and Analysis Committee of the American Oil Chemists'

Society, and as such have had much to do with the detail of the procedures with which the cottonseed grade has been provided to automatically adjust prices for minor variations in quality of cottonseed as marketed throughout our country. At present we try to provide for variations in oil quality by measurement of the free fatty acids in the oil and in the seed. It would be very simple to put in some sort of colorimetric measurement of an extract of oil and to relate this empirically as an additional factor in the cottonseed grade. I am sure that this would determine the degree to which the glandless seed was appearing in our trade. The proposal for such a change should originate with the industry management.

V. L. Frampton: The question was raised about the usefulness of available lysine procedure for stimulating the quality of the meal. I agree completely with Professor Watts in his comments, and I want to repeat what he said on Wednesday that where we fed, in cooperation with Dr. Watts, a series of meals of uniform available lysine content, there was a variability in the response of these animals and birds that up to the present time is unaccounted for. We cannot use the available lysine alone as a single and simple measurement of protein quality. There is something else of importance nutritionally in these meals that we have yet not identified.

G. D. Kapsiotis: Mr. Chairman, with your permission, I would like to go back to the methods of producing cottonseed flour. We have known for a long time, of course, of Proflo. We have known of the consistent quality of this product; we have tested it in a number of countries, in the Far East, and in the Middle East, but nowhere have we seen a description of the processing method. And then there is the rather high price that the purchaser has to pay to obtain the Proflo cottonseed protein concentrate. Mr. Cochran has indicated that the method is available, has been described and can be utilized in any country where cottonseed exists. Is this so?

G. B. Cochran: Company policy will not permit me to give any details on our process. Whether it can be used in other countries we do not know. We have never attempted to manufacture flour overseas.

I will comment on price. The cost of our flour seems to be the general bug-a-boo. As I sat down a minute ago, Dr. Milner said to me, "We are not going to pay fourteen cents a pound for Proflo." This points out one of the problems when people talk about our price they talk in one-bag lots. I never hear anyone talk about carload quantities which are quite inexpensive in comparison. We will be glad to lower our prices if someone wants to buy in quantity but when you deal in one-and five-and ten-bag quantities at a time, I think most commercial people will admit that costs go up considerably. There is another problem which adds considerably to any food processing cost here in the United States. That is processing to standards that meet the FDA's requirements. On top of that, we must guarantee a free gossypol content of less than 450 parts per million. We have laboratory facilities to insure that every lot of Proflo meets these requirements, so this again adds to the cost, though basically, our cost f. o. b. Fort Worth is about ten cents a pound. The problem of getting it overseas is one of transportation and we find that to be quite expensive. By the time it arrives overseas another two cents is added; so this makes the price about twelve cents per pound.

Max Milner: Mr. Chairman, I wonder if Dr. Altschul would also agree that one omission in his summary was that section of the program provided by Barlow, Bitting, and Bean. That is the need for more attention to the economic aspects in the utilization of oilseed proteins. I think this is a very important new element in conferences of this kind. I feel that particularly valuable is Dr. Bean's appeal that economists and marketing specialists ought to be brought more closely into the work of the technologists. This will have to occur before a really rational development will take place in this field.

C.M. Lyman: It has been the tradition of those who have met from time to time for quite a number of years now to express our ideas. Well, where do we go from here? Dr. Altschul has done an excellent job of summarizing this morning. I would like to point out one area in which it seems to me we need to be looking forward. I think it is quite evident that at least for the first few years the use of glandless cottonseed will be of the greatest benefit in the production of protein for human use. Isn't it time now to be thinking of a program some place in the world where we need this protein for human use? Isn't it time to think of an organization by which we can get the seed planted, by which we can process the seed and study the economical gains to be obtained by using this type of seed? Maybe I am wrong. Maybe this thing will come about more or less automatically, but I am intrigued with the possibility of a pilot program for evaluation on production scale, a pilot program in an area where we need proteins for human use.

B.H. Wojcik: Dr. Milner, you might have something to say on that subject.

Max Milner: I do believe that in certain aspects, UNICEF and FAO are very much interested in this kind of an approach. I am wondering if Professor Wamble or Layton Allen might not like to make some announcements in relation to a project which has recently been undertaken at the Texas station to try to establish more firmly some of the answers to technological problems of the kind we have discussed here.

A. C. Wamble: Well, I had hoped you would call on Garlon Harper again. I think you all know that these folks have some plans in the not too distant future to process some of this seed. I will say just a few words about our work with UNICEF. We are working very hard over at Texas A&M right now trying to come up with some method, maybe more than one, that within the very near future can be used in some of the developing countries. We are working with the idea of getting something like Proflo or maybe a slightly superior product that can be produced at a very early date. We are also working along lines that have been reported to you on the possibility of actually extracting all of the gossypol along with the oil.

One other idea that we are toying around with is getting part of the gossypol out with something like the expeller operation to take care of those cottonseeds that exceed the one percent gossypol level to start with. In fact, we have demonstrated on a small scale that you can actually squeeze out about a third of the gossypol in cottonseed meats if you properly prepare them for prepressing. The drawback to this particular procedure is that if you do this, you don't bind up the gossypol that is left as well as you would like. To accomplish that job, you might have to give it some additional heating later on. We are not too sure about this because the work we have been doing has been done on a pilot expeller, and our experience has been that the larger expeller, like the ones used commercially, do a much better job of binding gossypol than the small one we are using. We are therefore not ready to give answers until we can talk somebody into doing this on a commercial scale. Fortunately, we have recently learned there is a mill whose normal method of processing is something not too far from this procedure, and we are beginning to look into that operation a bit.

We have made some progress in improving the preparation of cottonseed meats prior to prepressing or hard pressing or direct solvent extraction. The procedure we use is not the one that was used to produce the meals Dr. Watts spoke of. The procedure we used in the case of the meals Dr. Watts spoke of was somewhat of a compromise because at the time we wanted to use the same type of procedure that the Southern Division was going to use in connection with their mixed solvent process, that is in the heating part. Those meals were not as low in free gossypol as we would like for them to be. Since then, we have used different procedures that have lowered the gossypol without seriously affecting the protein solubility. We have not yet nutritionally evaluated these meals, but this will be done shortly by people the National Cottonseed Products Association is lining up. Dr. Watts is among them I am sure.

Just to give you a rough idea of the procedure involved, we start out with seeds a bit drier than one usually wishes to flake, and we try to build the moisture content up to at least nine percent prior to flaking the material because of the obvious difficulty we might have in rolling them. Now, we put these seeds in the cooker and immediately start using a generous supply of live steam. In other words, the cooker is steam jacketed; it is just a smaller size cooker built very much like the commercial cookers; and we also use a generous supply of live, saturated steam directly in the meats for two purposes -- to help build up the moisture content and, of course, to get the material heated as rapidly as possible. Now, in addition to this live steam we immediately add at a fairly rapid rate, enough moisture to build the moisture content up to somewhere between 11 and about 14 percent. We won't know until we get our evaluation on these materials exactly what the optimum point is, but within that range, the meals have good chemical characteristics. By that I mean the protein solubility is good and the free gossypol is fairly low. I think the secret to the whole problem of maintaining this protein solubility is that after about 20 minutes of this type of treatment we cut off the live steam and allow the material to dry in the cooker only a sufficient length of time to reach a temperature high enough that we can take the material out of the cooker and run it over a screen and flush off the remaining moisture. We are not using any extra air in our pilot plant because we handle the materials by hand, but I believe a commercial plant would probably want to pull a generous supply of cool air over this screen that is used for two purposes, one of which flash cool this material, the other is to dissipate the moisture. It is advisable to remove all the moisture you can under these conditions down to as low a temperature as you can, and at the same time take out any water balls. After the water balls are removed they are ground and run through a sort of gentle grinding process. They are then brought back over the screen to cool them down a bit. This cooling stops some of the heat damage that you normally would have if you cook in the usual methods that most people use. Now, to go on from there, we get very good results on direct extraction of material prepared in this manner. While we haven't tried it on a large scale yet, we have reason to believe that the meal would probably extract sufficiently well, though a solvent plant might have to reduce its production slightly because of the extra oil which would have to be extracted, but it really comes out easily.

Now then, if you are going the other route, and either prepress or hard press, this material is reheated to just as low a temperature as you can afford to use and reduce your residual oil in your material to a satisfactory point, but do this as quickly as possible through some sort of tempering device. The usual type used in connection with the expeller and screw press would probably be satisfactory. Actually, for human food uses you probably would want to try a process somewhere between prepressing and full pressing and reduce the oil content low enough that you would not have any problems in grinding and screening the meal down to the finest that you would want.

Now, as I have indicated this is all preliminary information. A lot of our evaluations have not been made. No biological tests have been made on any of these meals to see what that protein quality is, but to give you an example, recently we used a process somewhere intermediate between prepressing and hard pressing and produced meals that had a solubility of about 70 percent. The gossypol was quite satisfactory. The only thing that was a bit off was the oil content, and that particular meal was about seven percent. But this again was only a small scale expeller and all of our experience in the past has indicated that materials handle better, the oil content comes down lower, and the gossypol binding is a bit more complete in the larger machine. We would expect that with commercial equipment the solubility might be reduced slightly, but probably not much.

B. H. Wojcik: I think at this time I would like to give the man that started all this, Dr. Altschul, a chance to say something, and then after that, Garlon Harper, I would like for you to make some remarks; and Layton Allen, how about you?

A. M. Altschul: I will not attempt to answer the questions raised; the point to my talk was to stimulate discussion, and apparently it did.

G. A. Harper: We had an opportunity earlier in this Conference to thank everyone for their attendance, and anticipated participation, in behalf of the National Cottonseed Products Association and the Cottonseed Crushing Industry as a whole. Now it is a very great pleasure indeed to express appreciation to Aaron Altschul for the tremendous job which he has done in getting this program together. I think all of you know that the mainspring back of this Conference was Aaron Altschul; he has certainly done a wonderful job as have all of the chairmen and all of those who participated in the program and presented papers. On behalf of the National Cottonseed Products Association I thank all of you who attended and were so interested in the presentations which were made and the discussions which followed those presentations. It is a great pleasure to express our appreciation to you.

B. H. Wojcik: Layton, how about you?

L. E. Allen: On behalf of UNICEF I too want to express our appreciation for the participation of all of you in this Conference. It is important to us because the deliberations at this Conference will serve as our guidelines in pursuing our program of feeding hungry children, and that is why UNICEF was interested in being a cosponsor of this Conference.

Friday Luncheon Session
January 17, 1964

Karl Mattil: Our speaker today is going to talk on a subject that is very important to us. Many of us in the scientific field are concerned only with the subject, can we make it? This is only part of the problem, and often it is the easier part. A more important part of the problem and one that should be considered early in the game is, can we sell it, because if it can't be sold, why make it? Our speaker today is in the business of selling these things that we try to make. He has a real story to tell of a product in which we are interested. I am hoping that some of you who are more interested only in the aspect of can we make it, will listen to his story of can you sell it?

Our speaker Mr. Phillip Henry Hind is a native of Durban in South Africa. His father was a pioneer in the food industry in South Africa, establishing their company in the late nineteenth century. He is now the manager, director and chairman of that company, the Hind Food Company. Mr. Hind.

INTRODUCING A NEW PROTEIN-RICH FOOD (THE MARKETING OF "PRONUTRO" -- A HIGH PROTEIN FOOD)

by

P. H. Hind
Hind Bros. & Co., Ltd.
Durban, South Africa

Every story must have a beginning, and this story starts in 1937 when Doctor C. W. Fox, who at the time headed the Biochemistry & Nutritional Division of the South African Medical Research Institute in Johannesburg, called to see me. He was worried at what he had seen on a recent visit to the Transkei on South Africa's eastern coastline where African children were literally dying by the thousands owing to malnourishment, and asked whether our company could help to solve the situation by marketing a baby food at a price to meet the purchasing power of the lower income group. I suggested his approach should be to milk processors, as at that time the medical profession considered animal proteins were of infinitely more value than vegetable proteins. Being cereal manufacturers ourselves, we had no access to a cheap source of milk. Dr. Fox mentioned that he had already made such an approach, but due to the high cost of milk, manufacturers were unable to market a milk powder at a lower price than the prices prevailing at the time.

We pondered over this problem and decided that the only solution was to provide a cereal food and to include defatted soya bean meal to bolster the protein value. To ensure that this food would have little effect on the sale of our other baby foods, which sold primarily to the white population, and contributed largely to the overall profit of our business, we decided to call it by a Zulu name--"Incumbe". (Meaning a maize gruel prepared by the Zulu mother to feed her baby.) We also decided that the label would be printed showing an African baby; and we confined feeding directions to four African Languages, eliminating English and Afrikaans languages from the label entirely. Thus was born Incumbe, a product which over the years has proved a good seller, and has certainly played a part in stemming to a certain extent, malnutrition among the lower income groups.

Our policy in marketing Incumbe ran along the lines that the consumer price should be fixed so as to meet the purchasing power of the lower income groups. To do this meant sacrificing profits, relying on greater output as the sole means of reducing overhead, a policy which we have followed throughout.

I advised Dr. Fox that we would make every effort to introduce a milk powder into the Incumbe formula as soon as we were able to find a cheap source of milk supply. The War years, of course, intervened, and it was not until 1947 that an opportunity presented itself to purchase the controlling interest in Powdered Milk & Dairy Products, a milk powder factory situated in the Orange Free State area. As a result an immediate change in formula was made in which milk powder was added as an ingredient to Incumbe.

Incumbe sales continued to increase until 1956 when they started a slow decline. In analyzing the situation, the following factors emerged. The negro was becoming more sophisticated, and thus suspicious of a food, or for that matter any other commodity, made specially for him. He believed, quite understandably, that such articles must be inferior, and also tended to believe that the white man was trying to profiteer at his expense. We had to admit that we had not really convinced the medical profession that Incumbe was the ideal product. Our choice of the name Incumbe rebounded as it was unfortunate that many negro mothers when taking their sickly children to a clinic on being questioned as to the food given would reply "Incumbe". The large majority of Doctors quite naturally drew the conclusion that it was our food whereas, in fact, it was the mothers' own gruel made from maize.

Still convinced of the urgent necessity for a suitable food and the great potential offering to the successful manufacturer, it was decided to reopen research to find a product that would be acceptable to the medical profession as well as to all races. Such a food would have to be made from materials readily available at low cost, and if possible would also be one of convenience--i. e., precooked. Cooking is not only an added expense, but among the more primitive people using open fires it is a dangerous hazard with children playing about. This, therefore, was the directive given in 1957 to Doctor E. A. Waldburger, our Company's Chief Research Officer.

Product Research

To convince the medical profession, Dr. Waldburger set about his task of compounding a food which would have as near as possible the identical amino acid breakdown and biological value as found in fresh skimmed milk. Dr. Waldburger also took cognizance of UNICEF's recommendation that protein enriched foods should be prepared mainly from waste products of industry, such as oilseed cake, cottonseed flour, skimmed milk powder, defatted soybean flour, thus keeping down raw material costs to a minimum. Finally, he developed a formula giving the required results in which the following raw materials were used:

Skimmed milk
Defatted peanut meal
Defatted soya bean meal
Fish flour
Food yeast
Wheat germ
Bone meal, with added iron
Iodized salt
Vitamin B
Sugar

These first efforts, however, were thwarted by the early onset of rancidity, which problem he finally overcame enabling us to approach Doctor Frank Walt, one of Durban's leading pediatricians, to test the product.

Dr. Walt, who shared the views of many of the medical profession that vegetable proteins were inferior to animal proteins, was persuaded largely on account of its composition, and its comparative high biological value of 72, to try the food at McCords Zulu Hospital, Durban, where he ran parallel tests on African babies suffering from kwashiorkor. Half of the patients were fed on skimmed milk powder and maize--the recognized cure--while the other half were given a mixture of ProNutro and maize. The results of his work appeared in the British Medical Journal of January 13, 1962, Volume 1, pages 73-75. ProNutro at this time was known as "NUTROFOOD", but we later changed to "PRONUTRO" as the majority of doctors felt it a more suitable and apt name. These trials showed the product up in a very favorable light, and currently with Dr. Walt's experiments, similar trials were undertaken by the Medical School of the University of Natal at King Edward VIII Hospital, Durban. Once again the results proved satisfactory.

We decided at this stage to invite Dr. W. A. Odendaal, a well known nutritionist, who at that time headed the Nutritional Department of the Council for Scientific & Industrial Research, to visit Durban and to see for himself the results already achieved. Dr. Odendaal was impressed--so much so--that he agreed to lend his support to our efforts and joined our staff as nutritional advisor. He felt that several improvements could be made to the formula, and suggested that we replace peanut meal with whole peanuts--his reason being that care must be exercised to ensure that only fresh peanuts be used as old peanuts could have a toxic effect, also by using whole peanuts we would improve the calorific value. He also suggested using whole soya beans, and to offset the increased cost of using whole peanuts and whole soya beans he recommended reducing the skimmed milk solids to 10 percent, and adding 6 percent whey powder. These changes presented many problems--such as early rancidity, and the poor absorption of proteins. Finally, these difficulties were overcome and the new formula was ready for testing.

The Natal Medical School was again most cooperative in carrying our further experiments on kwashiorkor cases. The results of this work appeared in the South African Medical Journal of October 7, 1961. Field tests were also conducted at Lamontville, an African township near Durban, in which 1,200 children ranging from 6-1/2 months to 3 years were given 2 ounces of ProNutro per day as an additive to their normal maize diet. The results--no deaths, no cases of malnutrition, and an average weight gain double the normal. It should be borne in mind that unfortunately the infant death rate is normally 270 per 1,000. Work on children outpatients was also conducted at Meadowlands in the Transvaal. The reports on this work appeared in the South African Medical Journal dated December 1, 1962. Finally, at the request of Professor J. D. L. Hansen of the Department of Child Health, University of Cape Town, clinic tests were conducted on kwashiorkor patients in which the amount of protein was reduced by half to one gram per pound body weight. The results of this experiment showed that ProNutro can cure kwashiorkor at moderately low levels of intake, but the response is slower than with the equivalent amounts of skimmed milk.

To ensure ProNutro's general acceptability by nutritionists, we approached the South African Nutrition Society requesting that papers outlining the work done by five Doctors on ProNutro be read at the Nutrition Conference which was to be held in Cape Town in February 1962. Permission was granted and the conclusion reached was that ProNutro was a valuable high protein food, and that it can play a very useful part in the prevention of kwashiorkor at a cost of just over 1 cent per child per day in those areas where milk is scarce and expensive. With this assurance that the medical profession had accepted ProNutro as being nutritionally sound and well balanced we felt that the time was now ripe to consider the introduction and marketing of the product.

I must at this stage apologize for this lengthy introduction, but to successfully market a product to combat malnutrition, the first essential is to ensure the backing of the medical profession and to those men and women who worked on our behalf we are indeed grateful. In appreciation of this gratitude we assured them that if the food was a success it would be made available at cost to all charitable organizations interested in child welfare.

Market Research

Due, of course, to our earlier experiments we were assured that ProNutro was acceptable to both European and Non-European babies. An important point to note at this stage was that in 1957 an experiment was conducted in Durban in which African workers in five of Durban's biggest factories, all of whom ran their own canteens, were examined by a team of Doctors. The results indicated that nearly all were suffering in varying degrees from malnutrition. This finding sparked off a new idea. If ProNutro is good for children surely it should be equally as good for adults. This thinking certainly helped our marketing thoughts as it meant a far greater potential market than was first envisaged. We realized, of course, that to obtain adult acceptance, created a further problem--namely--that of educating Africans to accept a new type of food--no easy matter--but certainly a worthwhile challenge.

Our first trial efforts were carried out at the Dunlop Factory in Durban. The purpose was to ascertain the reaction of 32 adult Africans to the taste of a soup made with ProNutro and hydrolized protein paste. Immediately before serving, these two ingredients were added to the usual vegetable and meat bone soup. The ProNutro acted as a thickening substance in place of peaflour, and the hydrolized protein paste gave added flavor. On the first day the 32 were given this soup free of charge. On the next four days they were charged 1 cent per cup and only one did not buy; the other 31 bought three or four times. The news of the nice tasting soup spread throughout the factory and the person conducting the experiment had difficulty in refusing the other workers who also wished to buy the soup. This was an encouraging initial response, indicating that ProNutro as an additive to soup or stews or gravies was possible.

Further experiments have since been carried out in which ProNutro was used as an ingredient in Mahewu--a sour, unfermented drink made from maize which has national acceptance by the African population. The results again were found to be reasonably encouraging. These trials provided sufficient evidence that ProNutro was acceptable to male Africans, although we realized that unless we could convince them that ProNutro was being eaten by white adults, little success would be achieved. Experiments were therefore conducted among white school children, adolescents, and elderly people of both lower and higher income groups.

Dealing with school children and adolescents we were successful in obtaining the cooperation of Robert Campbell--an Australian professional swimming coach who had recently arrived in this country. He was amazed to learn that coaches in South Africa paid little attention to their charges' diet. Learning of the ingredients in ProNutro he showed immediate interest, stating that it contained all the essential ingredients which he had been recommending separately--i.e., peanuts, soya beans, food yeast, wheat germ, etc. He immediately commenced recommending ProNutro to all his pupils as an essential part of their diet. A team consisting of three Doctors and Mr. Campbell then arranged to test a group of Rugby and Soccer players, athletes--including long distance runners--swimmers, golfers, tennis players, and others, on the following meal taken approximately three hours before actually participating in their respective sporting events:

ProNutro	1 cup
Sugar	4 dessert spoons
Fruit juice	The equivalent contained in two oranges.

Stirred in cold or lukewarm water to the desired liquid or semiliquid consistency.

This quantity was recommended for youngsters of approximately 12 years and 1-1/2 to 2 times the amount was taken by older and heavier athletes. No untoward reactions were encountered, and because of the success achieved in many instances, particularly insofar as increased stamina was concerned, the tests were considered successful. There was no feeling of fullness, and no hunger pangs were experienced, while all who participated stated that the mixture was palatable.

We believed that we now had sufficient evidence to test market the product to ascertain whether the lower and middle income groups would be interested in buying the product, and whether it would be acceptable to elderly people. We decided to introduce ProNutro in a 1 pound packet selling at a consumer price of 15 cents. To achieve this low selling price we were compelled to reduce the wholesale and retail profit to 7-1/2 and 15 percent respectively--the normal markup being 10 to 15 percent wholesale and 25 to 33-1/3 percent retail. To obtain the support of the trade, we included a "pack in slip" with the following message:

> "This product is brought to you at this low price by the cooperation of Manufacturer, Wholesaler and Retailer who have reduced their normal profit margins."

The trade in general was not keen to handle the product at such low margins, in fact many refused to carry the line. We did obtain support of a limited number of Traders, and the experiment provided us with much useful information. It indicated that people were prepared to purchase and repurchase the product, and as this acceptance came from a cross section of the public it indicated possible success if ProNutro was properly marketed.

Marketing

In attempting to launch ProNutro to the South African public, it was decided to improve the packaged design, slanting it towards an image for the white market, and to also extend our range by including a 2 ounce and 4 pound pack. It was evident that if we were to be successful we had to allow the trade their normal profit margin, and a reasonable sum of money would have to be made available for advertising. It was therefore decided to increase the consumer price of the 1 pound packet which previously sold at 15 cents per packet to 20 cents per packet, thus giving a reasonable profit margin. At the same time we introduced a 4 pound pack selling at a consumer price of 60 cents per packet or 15 cents per pound, thereby continuing to make available to the public, ProNutro at 15 cents per pound. The 2 ounce pack sold at a price of 3 cents. ProNutro at this time was being offered in 40 pound polyethylene lined paper bags to child welfare organizations, etc., at 8-1/2 cents per pound, which price was actually below our total cost--while industrialists were quoted 10 cents. As a matter of interest and for a comparison--skim milk sells at 15 cents per pound, while a 2 pound loaf of bread costs 9 cents.

On the new pricing structure it was envisaged that a sum of R25,000 was the minimum required to do a national advertising campaign. To initiate the launch, we held a press conference in Durban and outlined the research work which had been undertaken, the success so far achieved in overcoming malnutrition, and at the same time highlighted the work done in using ProNutro as a pregame meal. We followed this up with a luncheon on the same day, to which leading nutritionists and others interested in welfare work were invited. A second lunch was held a week later in Johannesburg. The free editorial publicity obtained was tremendous. Articles appeared in twenty-four papers circulated throughout South Africa. In the "Transvaler", the main Afrikaans language paper in the Transvaal, we got a bold heading on the front page over five columns. The "Landstem" which is the leading Afrikaans paper in the Cape Province gave us front page news, while the "Natal Mercury" (English language) in Natal printed a front page spread with large headlines over four columns, the article extended to the second page. The total readership, in both languages of the twenty-four papers concerned amounted to 5,297,000. In viewing this figure it is interesting to note that the total white population of the country is 3-3/4 million. One of the reasons for the enthusiastic response from the press was that the United Nations, with whom ProNutro had previously been discussed, recognized the product as a valuable food which could play a part not only in helping to overcome malnutrition in South Africa but in other parts of the world.

A reasonably good distribution of ProNutro was then effected, supported with leaflets at point-of-sale and direct mail to the medical profession. In addition, a press campaign was scheduled at a cost of R17, 000. The advertising theme was based on the realization that in our busy, modern way of life, breakfast--a most important meal--is skimped. Thus we considered our approach to marketing ProNutro be centered around the theme "The Complete Breakfast in a Plate," in which the nutritional value of a plate of ProNutro was compared with the nutritional value of a good standard breakfast. This was to be our main approach.

The results were somewhat disappointing when viewed on a national basis. However, sales continued to grow in Natal, mainly in Durban and environs where much of the research work and field trials had been carried out. In Pretoria in the Transvaal sales also responded reasonably well--again due to the early research conducted on the pregame meal. The other areas showed very small gains. It was therefore decided to discontinue advertising after we had spent R12, 000. This was in February of 1963. From February through to June further consideration and planning was given to devising an advertising campaign which would create an interest primarily among the whites. At the beginning of July the new marketing plans were presented and approved, and an amount of R100, 000 was earmarked for advertising and publicity over the following twelve months. The basic thought behind the new campaign was to create the image that ProNutro was a food for all, and that its sales success was due to the fact that the white population were purchasing it in ever increasing quantities. In fixing a figure of R100, 000 for advertising we estimated we could sell during the year 2, 800 tons, which would provide a profit without advertising of R100, 000. In other words, we were satisfied to cover all costs and to plough back during the first year the anticipated profit. It is interesting to note, that of the 2, 800 tons, we estimated supplying welfare organizations with 400 tons.

Advertising Platform

Having in ProNutro a unique product with all the attributes of balanced nutrition and the backing of the medical profession, we now had the platform on which to build a story of excitement. However, our problem was to condense this extremely long story into a graphic and compelling advertisement. It was finally agreed that our introductory advertisements would take the form of editorial news-type pages with subheadings which would attract all sections of the community--i. e. , "ProNutro Acclaimed by Sportsmen," "South African Scientists Develop Wonder Food," " School Teachers Praise ProNutro," etc.,

Some headings in advertisements were capped by exciting headlines--i. e. , "The Greatest Advance in Nutrition since the Discovery of Bread." The copy was based on health, with balanced nutrition being the main theme carried throughout. To more strongly emphasize the "balanced nutrition" aspect a scale was introduced into the advertisements which were in two colors--the scale being the only part of the advertisements printed in red. The scale, we felt, would symbolize the product, and would eventually gain for ProNutro a very positive identification. The theme and image was "Health through balanced nutrition" with a slogan--"A perfect way to a healthy day."

It was agreed that the press campaign should be divided into four distinct phases:

Phase 1: Full page advertisements were placed with the exciting headlines--"The Greatest Advance in Nutrition Since the Discovery of Bread" in which we, the manufacturers, acclaim ProNutro, the "Wonder Food."

Phase 2: We decided that as we the manufacturers had told the public of the attributes of ProNutro we should now tell them, in a recommendation type of advertisement, what they, the public, are saying about the product. This advertisement, therefore, had a headline--"South Africa Acclaims Wonder Food." Again the entire advertisement was split into exciting subheadings to attract all sections of the community. The scale in red was retained, and the payoff slogan--"A Perfect Way to a Healthy Day"--was continued.

Phase 3: Confident that the product would be nationally acceptable and that sales would soar, we now showed the public the sales climb in graphic form and backed this by a statement emphasizing that such heights, through public approval of ProNutro, had been reached. The heading--"Continued Amazing Success of ProNutro--The Wonder Food"--was followed by a subheading--"Sales Rocket as People Prove the Worth of this 'Wonder Food'." The main body of the advertisement showed a graph illustrating the steep rise in sales from June 1962 to September 1963. The body copy was condensed giving emphasis to the fact that ProNutro was tested and approved and also that so many sportsmen and athletic coaches, mothers, men and women of all ages from all walks of life had written sincere letters of gratitude and appreciation to the manufacturer of this 'Wonder Food' for the benefits derived. Both the second and third phase advertisements carried a coupon which could be sent in for free literature as well as a free recipe book.

Unfortunately the demand for ProNutro outstripped supply and while additional manufacturing equipment was being installed, we discontinued advertising after only one or two advertisements in third phase had appeared. As soon as these factory problems are overcome the third phase will continue.

Phase 4: This campaign is designed to emphasize to the consumer the fact that ProNutro is acceptable in all walks of life, by all ages, and by all people doing various jobs of work and playing different sports, etc. This campaign is again based on a recommendation type advertisement. (Unsolicited testimonials will be used.) The main body depicts men and women recommending the merits of ProNutro. The scale in red, and the slogan--"The Perfect Way to a Healthy Day"--are maintained. Every third advertisement in this phase is designed to show a housewife in the kitchen using ProNutro in various ways. These recipes were designed to teach the public of the many uses to which ProNutro can be put. The final phase of the campaign is to be the backbone of all future ProNutro advertising, for the time being.

Radio: In order to give the press and magazine advertising additional support it was decided to use radio for a period of three-and-a-half months and the National Springbok network carried half-minute and quarter-minute spots in the early and late morning time channels, and in addition, a midmorning 15-minute program called "Front Page Story" which dramatizes great events of the past. This time channel carries an opening forty-five second and closing forty-five second commercial.

Merchandise: Point-of-sale material helped give the initial launch greater impact and the following point-of-sale pieces were designed:

- Store banners
- Window bills
- Dump bins
- Wobbler flags
- Recipe books

All sales representatives were issued with a "flip flop" sales aid promotional booklet so that they could visually illustrate to buyers the product description, the volume of advertising, the potential sales, together with the profit margin offings.

It was thought essential that the public be given every opportunity of learning the various ways ProNutro could be put to use, and some 100,000 recipe books were printed of which 25,000 were distributed at store level. These were placed in specially designed containers which were fitted to all dump bins at point-of-purchase.

Field Work--Public Relations

Since ProNutro had gained the support of the medical profession it was decided to further establish the product by employing a health educator to address organizations such as Women's Institutes, Welfare Organizations, Missions, Rotary Clubs, Health Institutions, Red Cross, etc. This campaign has proved most successful. Along with this work we have employed another health educator whose responsibility it is to promote ProNutro to industry, mining groups, and government agencies, many of whom are today including the product in their canteen diets.

As stated earlier, it was the intention to market ProNutro in the first year directly to the whites, but at the same time it was felt necessary to promote ProNutro directly to the African, not through press and radio, but with African demonstrators who would visit clinics, mission stations, and African townships to give demonstaration of the product.

A flannelgraph was used, and the demonstrator unfolds his story by placing flannel figures on a board to which flannel is attached and to which the flannel figures adhere. The story commences by showing the African people in the early days, living on their farms with their fine physical condition and robustness and the food which they ate. It continues by showing the drift of these rural people to the cities where owing to the lack of knowledge regarding correct nutrition and their predilection for starchy foods and sweet mineral water drinks, physical deterioration sets in. The flannelgraph then depicts the ingredients and well-balanced nutritional value of ProNutro, and concludes by showing how the undernourished African regained his health and stamina by including ProNutro in his diet. The audience tastes the product and are given samples.

This service has since been extended by using a mobile van fitted with a public address system. The area chosen for the initial trial operation was the African territory of Zululand. Stores throughout this country are preselected for demonstrations. Prior to the arrival of the van and giving the flannelgraph talks, the demonstrator on an earlier date, places advertising material at the store and a placard indicating when the demonstration will be held. The advance contact is made approximately two weeks prior to the demonstration. On the selected day, and approximately three miles from its destination, the van moves slowly along the road, and the demonstrator announces that the show is on. The public address system has an amplifier with an audio range of 4 miles, thus all in the area can be contacted. At the scene of the demonstration, colored flags are set out to give an effect of gaiety, excitement and interest. By the time the demonstrators are ready to put across their flannelgraph and promote the merits of ProNutro, audiences up to 1,000 can be assembled. After the demonstrations, a selling scheme is conducted at the store whereby the African can purchase two pounds of ProNutro at a specially reduced price. It is the intention to expand this campaign throughout the other African territories in view of its success. A Cine Film showing such a demonstration in action is available for showing.

This now completes the story of the methods we have adopted in introducing ProNutro on the South African market.

For the 12 months ending June 1962 we sold 72 tons of ProNutro, for the year ending June 1963 we sold 723 tons, while for the first five months of the current year we have sold 872 tons, aiming as I indicated earlier on reaching a target of 2,800 tons for the year ending June 1964. We may seem a long way from this target, but we feel confident that by June of this year we should not be too far from our goal. Sales for December were averaging just over 63 tons per week.

On behalf of Dr. Waldburger and myself I would like to express our appreciation to the organizers for inviting us to attend this Conference which we have found not only interesting but most helpful. I therefore hope that what I have had to say today might also prove stimulating and helpful to you.

DISCUSSION

Karl Mattil: Thanks again to Mr. Hind and Dr. Waldburger. Here you see the end product of long years of research and development. To those of us in industry who are in research, this is the gratifying moment when the marketing people come back to say, "It is selling. It is a winner." Then you feel you have really made an accomplishment. Before we conclude, Dr. Milner would like to speak to you about a subject in which I think you people would be interested.

Max Milner: When we and our friends at the Southern Division were discussing the content of this program a number of months ago, Dr. Altschul asked: "Surely with all the interest in protein-rich food, something has been done, something has been accomplished which would indicate that there is some kind of a success story to tell." From this followed the presentation that you have just heard.

I think we ought to recognize that this is probably the most effective approach to solution of the problem of protein malnutrition in countries with semiliterate populations of low economic level. We suggest to you that here is an example to be emulated. I wish, however, we could say that this kind of enterprise is as prevalent as we would like it to be.

There are still many other problems that have to be solved in many countries. I am sure Mr. Hind could tell you the problems he had to overcome in Africa. This, of course, is the challenge to the United Nations, and to UNICEF and FAO particularly, in dealing with this question of how to introduce the commercial aspect of marketing.

I thought you might be interested in hearing today about three typical programs in which UNICEF and FAO are involved. Three programs in three countries are represented here. I will quickly run through them. The first deals with Senegal -- a mixture for preschool children. The amount of the UNICEF expenditure in this case is $65,000. This uses millet which happens to be the major cereal staple of the country, supplemented with local peanut flour, and for use by young children, some supplementation also with milk. In the latter case, the product will be what is called in North Africa, "cous-cous". The cereal (or the supplemented mixture) is cooked and then dried much like a cooked dried farina or corn meal mush. The objective is to demonstrate the feasibility of establishing permanent production and distribution channels, primarily commercial, for a nutritious food supplement for preschool children, that is, one to six years of age, with emphasis on the one to four age group. This cannot be emphasized too much. UNICEF is glad to be involved in school lunch programs of course, but the major challenge everywhere is to reach the preschool child in the home because this is where the problem is acute. By the time they are six years old, they have either made it or they haven't. In Senegal the commercial firm involved is called Sopral. The project will involve around 5,000 children over a two-year period, each receiving 100 grams per day. The World Food Program is also part of the story. They are donating some of the materials, principally the sorghum or millet. UNICEF is providing the peanut flour.

In Brazil another food mixture for preschool children consisting of corn and soy flours involve the commercial firm Nestle. This project has exactly the same objective, to demonstrate a new low cost product approach for getting improved protein nutrition to preschool children and to establish the food in low cost marketing. In this case, UNICEF's costs are $82,000 and this will benefit 25,000 children for again two years. The plan, of course, is for the Government and UNICEF to cooperate with a commercial company which is making its own marketing effort. There would otherwise be no purpose to this exercise. Such foods must be established through commercial markets before they can become effective.

And then finally, there is a product in India involving the large commercial firm of Tata, through their oilseed processing division. A cereal-based food consisting of 60 percent wheat flour, 30 percent locally produced peanut flour and 10 percent dried skim milk will be manufactured in Bombay. It will be sold at a subsidized basis parallel to the Tata Company's marketing efforts. One hundred grams of mixture daily will be provided to at least 25,000 children over two years. The World Food Program is supplying for this period the wheat flour and skim milk. I think this indicates then, the way which international agencies will now go in an expanding effort. I thought you might like to hear of this, and again I commend to you the kind of enterprise that we heard about today. (NOTE: Since the meeting a decision has been made to postpone this project for two years due to unforeseen circumstances not related to the marketing of the product.)

Chairman Altschul: I want to thank all of you who participated, all of you who chaired the meetings, and all of you who attended.

I want to repeat Dr. Wojcik's invitation to any of you who care to come out and visit the laboratory this afternoon. We will be glad to see you.

I want again to express my thanks to Dr. Wojcik and to the members of his staff who did such a wonderful job of arrangements.

The meeting is now adjourned. Thank you.

Program

Fontainebleau Motor Hotel - Grand Ballroom
New Orleans, Louisiana

Wednesday, January 15, 1964	
8:30 a. m. Registration	
9:00 a. m. Welcome	C. H. Fisher New Orleans, Louisiana
Opening Remarks	George W. Irving, Jr. Washington, D. C. Grace A. Goldsmith New Orleans, Louisiana
Presiding: Layton E. Allen, United Nations, New York	
Problems and Opportunities in Utilizing Cottonseed Proteins to Meet World Protein Deficits	Max Milner United Nations, New York
Trends in the Cottonseed Processing Industry in the United States	Garlon A. Harper Dallas, Texas
The Possibilities of Oilseed Protein Concentrates for Human Consumption in the United States and for Export	Frank D. Barlow, Jr. Washington, D. C. and H. Wayne Bitting Washington, D. C.
Comments	Louis H. Bean Arlington, Virginia
12:00 Noon Luncheon	
1:30 p. m., Wednesday, January 15, 1964	
Presiding: M. L. Anson, New York, New York	
Anatomy of Cottonseed	J. R. Wall New Orleans, Louisiana
The Ultrastructure of Cottonseed	Lawrence Y. Yatsu New Orleans, Louisiana
Proteins of Cottonseed	Wilda H. Martinez New Orleans, Louisiana
Presiding: Ruth M. Leverton, Washington, D. C.	
The Role of Gossypol	Carl M. Lyman College Station, Texas
Factors Affecting the Nutritive Quality of Cottonseed Protein	Vernon L. Frampton New Orleans, Louisiana
Role of Cyclopropenoid Fatty Acids	Richard A. Phelps Dallas, Texas
7:15 p. m. Dinner - Carnival Suite	
Presiding: George F. Stewart, Davis, California	
Adventitious Toxic Factors in Protein Concentrates	Leo Friedman Cambridge, Massachusetts

9:00 a.m., Thursday, January 16, 1964	
Presiding: Nevin S. Scrimshaw, Cambridge, Massachusetts	
Use Status of Cottonseed and Other Plant Proteins	Harold L. Wilcke St. Louis, Missouri
Technological Aspects of Incap Vegetable Mixture 9 (Incaparina)	Ricardo Bressani Guatemala, Central America
Introduction of Protein-Rich Food Preparations in Colombia	H. W. Bruins Barrington, Illinois
Experimental Production and Testing of Cottonseed in Peru	Antonio Bacigalupo Lima, Peru and Carl Widmer Lima, Peru
12:00 Noon Luncheon	
1:30 p.m., Thursday, January 16, 1964	
Presiding: Leo A. Goldblatt, New Orleans, Louisiana	
Review of SU Mixed Solvent Extraction: Process and Properties of the Product	Vernon L. Frampton New Orleans, Louisiana
Comments	Henry L. E. Vix New Orleans, Louisiana
Review of the Vaccarino Process and Products	Carmelo Vaccarino Venetico Marina (Messina), Sicily
Comments	Stanley P. Clark College Station, Texas
Review of New Solvents Available for Oil Extraction and Meal Purification	John A. Price Linden, New Jersey
8:30 a.m., Friday, January 17, 1964	
Presiding: Bruno H. Wojcik, New Orleans, Louisiana	
Progress in Breeding Gland-Free Cottonseed	Charles F. Lewis Beltsville, Maryland
Properties of Gland-Free Cottonseed Products	A. B. Watts Baton Rouge, Louisiana
Presiding: Aaron M. Altschul, New Orleans, Louisiana	
What Needs to be Done	
General Discussion	
12:00 Noon Luncheon - Carnival Suite	
Presiding: Karl F. Mattil, Chicago, Illinois	
Introducing a New Protein-Rich Food	P. H. Hind Durban, South Africa
Comments	Ernest Waldburger Durban, South Africa

Attendance List

CONFERENCE ON COTTONSEED PROTEIN CONCENTRATES
January 15-17, 1964

Allen, Layton E., Sr. Eng., Food Cons. Div., UNICEF, United Nations, N. Y.

Anson, Mortimer L., Consultant, 111 East 36th St., New York 16, N. Y.

Bacigalupo, Antonio, Dean, Dept. of Animal Sci., Agrarian Univ., La Molina, Lima, Peru

Barlow, Frank D., Dev. & Trade Analysis Div., Econ. Res. Serv., U. S. Dept. of Agric., Wash., D. C. 20250

Bean, Louis H., Econ. Analyst, 3714 N. Randolph St., Arlington 7, Va.

Bitting, H. Wayne, Staff Spec. (Food), Prod. and Proc. Eval. Staff, Wash., D. C.

Blaylock, Lynn G., Dir., Animal Nutrition and Res., Int'l. Milling Co., 1200 Investors Bldg., Minneapolis, Minn.

Bressani, Ricardo, Asst. Visiting Prof., Dept. of Nutr., Food Sci. & Tech., Mass. Inst. of Tech., Cambridge 39, Mass.

Bruins, H. W., Mgr., Nutr. and Biochem., John Stuart Res. Labs., The Quaker Oats Co., 617 West Main St., Barrington, Ill.

Buchanan, Ben F., Mgr., Tech. Applics., Res. Centr., General Foods Corp., White Plains, N. Y.

Caridad, Carlos, The Nestlé Co., Inc., 100 Bloomingdale Rd., White Plains, N. Y.

Cavanagh, George C., Ch. Chem. and Dir. of Res., Ranchers Cotton Oil, P.O. Box 248, Fresno, Calif. 93705

Clark, Stanley P., Cottonseed Prods. Res. Lab., Texas Eng. Expt. Sta., Texas A&M Univ., College Station, Tex.

Cochran, Gene B., Mgr., Traders Protein Div., P.O. Box 1837, Fort Worth 2, Tex.

Cole, Ronald L., Campbell Soup Co., 100 Market St., Camden, N. J.

Coleman, W. T., Chief Chem., Paymaster Div., Anderson, Clayton & Co., Box 521, Abilene, Tex.

Contesse, Gustavo, FAO Expert in Peru, Apartado Posta 2036, Lima, Peru

Cooney, Patrick, Pres., World Traders & Invs., Food Techniques, Inc.-Foreign Sales, 685 South Carondelet St., Los Angeles 57, Calif.

Corey, Harold, Thos. J. Lipton, Inc., 800 Sylvan Ave., Englewood Cliffs, N. J.

Cravioto, Rene, Inst. Nac. Protecion Infancia, Mexico City, Emiliano Zapata 340

Daggy, Elmer, Sr. Nutrl. Spec., Inst. of Nutr., Corn Prods. Co., 717 Fifth Ave., New York 22, N. Y.

DeMaeyer, Edouard M., Secty., Protein Adv. Grp., WHO/FAO/UNICEF, Room 1843-A, United Nations Hqs., N. Y.

Durán, Jorge, Gen. Mgr., GRASAS S. A., Carrera 18 No. 10-65, Buga, Colombia, S. A.

Friedman, Leo, Assoc. Prof., Dept. of Nutr., Food Sci. and Tech., Mass. Inst. of Tech., Cambridge, Mass.

Goldsmith, Grace A., Prof. of Med., School of Med., Tulane Univ., New Orleans, La.

Graham, W. R., Dir. of Res., The Quaker Oats Co., 617 West Maine, Barrington, Ill.

Hammerle, Olivia A., Bur. of Comm. Fisheries, College Park, Md.

Harper, Garlon A., Natl. Cottonseed Prods. Assoc., 2400 Poplar Ave., Memphis, Tenn.

Hay, Charles, Anderson, Clayton & Co., Houston, Tex.

Hebo, Halfdan, Vice Pres., American Protein Corp., P.O. Box 1523, Grand Central P.O., New York, N. Y. 10017

Herman, Roger Lee, Fishery Biol., U. S. Fish & Wildlife, Leetown, W. Va.

Hill, Ronald A., Eng., UNICEF, United Nations, N. Y.

Hind, P. H., Hind Bros. & Co., Ltd., The Garden Factory, P.O. Box 2823, Durban, South Africa

Hopper, John H., Dir. of Nutr., Kellogg Co., Battle Creek, Mich.

Hutchins. R. P., Vice Pres., French Oil Mill Machine Co., Piqua, Ohio

Johnsen, Dale W., Exec. Vice Pres., Soypro Int'l., 4830 South Christiano, Chicago, Ill.

Johnson, R. A., Dir. of Res., Food Techniques, Inc., 998 Felipe Ave., San José, Calif.

Kaplansky, Zeev, Food Tech., T. J. Lipton, Inc., 800 Sylvan Ave., Englewood Cliffs, N.J.

Kapsiotis, George D., Food Tech., Food Sci. and Tech. Br., Food and Agric. Org. of the United Nations, Rome, Italy

King, R. R., Consultant, Robert R. King Assoc., Preston at Sycamore, Sherman, Tex.

Kovacs, L. E., Pres. and Gen. Mgr., Vitamins, Inc., Chicago 21, Ill.

Leverton, Ruth M., Asst. Adm., Agr. Res. Serv., U. S. Dept. of Agric., Washington, D. C. 20250

Lewis, Charles F., Geneticist, Cotton & Cordage Fibers Res. Br., Crops Res. Div., ARS, U. S. Dept. of Agric., Beltsville, Md.

Lyman, Carl M., Hd., Dept. of Biochem. and Nutr., Tex. Agric. Expt. Sta., Tex. A&M Univ., College Sta., Tex.

Marshack, Eugene, Eugene Marshack Assoc., 50 East 42nd St., New York, N. Y. 10017

Mattikow, Morris, Consulting Chem., 11 West 42nd St., New York 36, N. Y.

Mattil, Karl F., Assoc. Dir. of Res., Swift and Co., Exchange and Packers Ave., Chicago 9, Ill.

Meyer, Edwin W., Dir. of Res., Central Soya, 1825 North Laramie Ave., Chicago, Ill. 60639

Milner, Max, Sr. Food Tech., Food Cons. Div., UNICEF, United Nations, N. Y.

Moore, N. Hunt, N. Hunt Moore and Assoc., 4515 Poplar Ave., Memphis 11, Tenn.

Morrison, A. B., Hd., Vitamins & Nutr. Sec., Food & Drug Directorate, Ottawa, Canada

Pavillon, Francois, Ing. Consulting, 150 Blvd. Masrena, Paris, (12e) France

Phelps, Richard A., Asst. Dir., Res. and Educ., Nat'l. Cottonseed Prods. Assoc., 2400 Poplar Ave. (P.O. Box 12023), Memphis, Tenn.

Price, John A., Res. Chem., Tech. Inf. Div., Esso Res. and Eng., P. O. Box 51, Linden, New Jersey

Proctor, J. F., Nat'l. Dairy Prods. Corp., Res. and Dev. Div., Glenview, Ill.

Quinn, D. F., Food Techniques, Inc., 998 Felipe Ave., San José, Calif.

Reed, P. M., Nat'l. Dairy Prods, Corp., Res. and Dev. Div., Norwich, N. Y.

Reich, William, Assoc. Editor, Calif. Farm Reporter, Santa Clara, Calif.

Reid, Leslie M., Tech. Staff, Oil Mill Div., Buckeye Cellulose Corp., 7854 Bobolink Dr., Cincinnati 24, Ohio

Rusoff, Irving I., Beech-Nut Life Savers, Inc., Port Chester, N. Y.

Sachsel, George F., Battelle Memorial Inst., 505 King Ave., Columbus, Ohio

Scrimshaw, Nevin S., Hd., Dept. of Nutr. & Food Sci., Mass. Inst. of Tech., Cambridge 39, Mass.

Shanks, Ernest, Res. Chem., Western Fish Nutr. Lab., Cook, Wash.

Sharon, Hilbert David, c/o Dept. of Biochem. and Nutr., Texas A&M Univ., College Sta., Tex.

Shaw, Richard L., Econ. Adv., INCAP, Guatemala, Apartado Postal No. 1188, Guatemala City, Guatemala, C. A.

Shirley, D. A., Hd., Dept. of Chem., Univ. of Tenn., Knoxville, Tenn. 37916

Sidhu, G. S., Dir., Reg. Res. Lab., Uppal Rd., Hyderabad, No. 9, India

Sidwell, Virginia D., Human Nutritionist, Bur. of Comm. Fisheries, USDI, Tech. Lab., Box 128, College Park, Md.

Sims, R. P. A., Dir., Food Res. Inst., Canada Dept. of Agric., Ottawa, Canada

Smith, Allan K., Sec. Hd., Northern Reg. Lab., 1815 N. University, Peoria 5, Ill.

Smith, Leonard, Dir., Util. Res. Com., Nat'l. Cotton Council, Room 502 Ring Bldg., 1200 - 18th., N. W., Wash. 6, D. C.

Smith, Oak B., Dir. of Mkt. Res., Wenger Mixer Mfg., 1802 Fed. Res. Bank Bldg., Kansas City 6, Mo.

Smith, Walton, Ch. Eng., Southern Cotton Oil Div., 210 Baronne St., New Orleans, La.

Stewart, George F., Chairman, Dept. of Food Sci. & Tech., (Cruess Hall), Univ. of Calif., Davis, Calif.

Subramanian, N., c/o Vitamin and Nutrition Sec., Dept. of Nat'l. Health and Welfare, Tunney's Pasture, Ottawa, Ontario, Canada (Permanent Address: Central Food & Tech. Res. Inst., Mysore, India)

Thionville, Paul C., Chem., Thionville Labs., 4301 Laurel St., New Orleans, La.

Tümer, Vacit, Eng. in Chg., Turyag A. O., Izmir, Turkey

Vaccarino, Carmelo, Mgr., The G. & S. Vaccarino Co., Venetico Marina, (Messina) Sicily, Italy

Verdery, M. C., Anderson, Clayton & Co., P. O. Box 2538, Houston 1, Tex.

Waldburger, Ernest, Hind Bros. & Co., Ltd., The Garden Factory, P. O. Box 2823, Durban, South Africa

Wall, J.R., Asst. Prof. of Biology, La. State Univ., New Orleans, La.

Wamble, A. Cecil, Hd., Cottonseed Prods. Res. Lab., Texas A&M Univ., College Sta., Tex.

Watts, A. B., Prof. and Hd., Poultry Sci. Dept., La. State Univ., Baton Rouge, La.

Wenger, Joe W., Wenger Mixer Mfg., Sabetha, Kan.

Wilcke, Harold L., Vice Pres., Ralston Purina Co., Checkerboard Square, St. Louis 2, Mo.

Wilding, M. D., Swift & Co., 4115 Packers Ave., Chicago 9, Ill.

PERSONNEL IN ATTENDANCE FROM SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

Altschul, Aaron M. (Dr.) - Seed Protein Laboratory

Bergquist, Jack J. - Administrative
Blair, Margaret M. (Mrs.) - Seed Protein Laboratory
Bourdette, Vernon R. - ARS Inf. Div.
Brown, Lawrence E. - Oilseed Crops Laboratory

Carter, Fairie Lyn - Oilseed Crops Laboratory
Connor, Mary Louise (Mrs.) - Seed Protein Laboratory
Cross, Daniel E. - Engineering and Development Laboratory
Crovetto, Albert J. - Engineering and Development Laboratory
Cucullu, Alva F. (Mrs.) - Oilseed Crops Laboratory

D'Aquin, Esler L. - Engineering and Development Laboratory
Deobald, Harold J. (Dr.) - Food Crops Laboratory
Dollear, Frank G. - Oilseed Crops Laboratory
Dupuy, Harold P. (Dr.) - Oilseed Crops Laboratory

Feuge, Reuben O. - Oilseed Crops Laboratory
Fisher, Charles H. (Dr.) - Director
Frampton, Vernon L. (Dr.) - Oilseed Crops Laboratory

Gastrock, Edward A. - Engineering and Development Laboratory
Goheen, Gilbert E. (Dr.) - Director's Office
Goldblatt, Leo A. (Dr.) - Oilseed Crops Laboratory
Gray, Marie S. (Mrs.) - Oilseed Crops Laboratory
Gros, Audrey T. - Oilseed Crops Laboratory

Hausser, Mary C. (Mrs.) - Director's Office
Hester, Opie C. - Economic Research Service
Hilding, Loeta B. - Director's Office
Hoffpauir, Carroll L. - Director's Office
Hogan, Joseph T. - Food Crops Laboratory
Hughes, James P. - Director's Office

Jones, Marie A. - Director's Office
Jones, Mary Alice B. (Mrs.) - Director's Office

Keating, Esmond J. - Engineering and Development Laboratory
King, William H. - Cotton Physical Properties Laboratory
Kopacz, Boleslaus M. - Director's Office
Kuck, James C. - Oilseed Crops Laboratory

Lambou, Madeline G. (Mrs.) - Engineering and Development Laboratory

Mann, Godfrey E. (Dr.) - Oilseed Crops Laboratory
Martinez, Wilda H. (Mrs.) - Oilseed Crops Laboratory
Mayne, Ruth Y. - Oilseed Crops Laboratory
Mazzeno, Laurence W., Jr. - Director's Office
Miller, Tahka E. (Mrs.) - Director's Office

Neucere, Navin J. - Seed Protein Laboratory

Pearce, Ellen S. (Mrs.) - Director's Office
Pepperman, Armand B., Jr. - Oilseed Crops Laboratory
Persell, Ralph M. - Director's Office
Piccolo, Biagio - Cotton Physical Properties Laboratory
Pominski, Joseph - Engineering and Development Laboratory

Richard, Grace M. (Mrs.) - Administrative
Rollins, Mary L. - Cotton Physical Properties Laboratory

Schultz, E. Fred, Jr. - Biometrical Services
Sharar, Beatrice A. (Mrs.) - Director's Office
Simmons, Joseph G. - Oilseed Crops Laboratory
Skau, Evald L. (Dr.) - Oilseed Crops Laboratory
Spadaro, James J. - Engineering and Development Laboratory
Stansbury, Mack F. - Director's Office

Verburn, Gerald B. - Oilseed Crops Laboratory

Vix, Henry L. E. - Engineering and Development Laboratory

Walker, Merlin H. - Plant Management
Williams, Nancy (Mrs.) - Economic Research Service
Wojcik, Bruno H. (Dr.) - Director's Office

Yatsu, Lawrence Y. (Dr.) - Seed Protein Laboratory

Zarins, Zigrida M. - Oilseed Crops Laboratory
Zeringue, Hampden J. - Oilseed Crops Laboratory

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, August 23, 1963

USDA to Sponsor International Meeting on Cottonseed Protein Concentrate:

An international meeting on cottonseed protein concentrates, sponsored by the U. S. Department of Agriculture, has been announced by Secretary Orville L. Freeman.

The conference, to be held in New Orleans, La., Jan. 15-17, will also have the support and participation of the United Nations Children's Emergency Fund (UNICEF) and the National Cottonseed Products Association. Dr. A. M. Altschul, Chief Research Chemist of the Seed Protein Pioneering Research Laboratory of USDA's Southern Utilization Research and Development Division in New Orleans, will be chairman of the conference. All three participating organizations will contribute to the program.

Attending will be representatives of U. S. and foreign scientific and academic institutions who have special knowledge of the basic composition and structure of seed, technical representatives of industry who are expert at producing high quality cottonseed products, and representatives of commercial firms, universities, and the United Nations who are concerned with bringing new foods and adequate diets to various areas of the world.

The technology of cottonseed protein concentrates will be emphasized on the program, Secretary Freeman said, with special stress on the preparation of high-quality proteins as human food.

Cottonseed is one of the principal crops being studied by UNICEF in connection with a worldwide program to supply additional protein for human diets in protein deficient areas.

The Southern Division is USDA's center for investigating the utilization of cottonseed, and carries on extensive research on its chemistry, processing, and utilization.

U. S. DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Southern Utilization Research and Development Division
New Orleans, Louisiana

FOR IMMEDIATE RELEASE November 7, 1963

Program Announced for International Meeting on Cottonseed Protein:

The program for a three-day international meeting on cottonseed protein concentrates in New Orleans, Louisiana, January 15-17, 1964, has just been completed, and includes prominent authorities on world food problems, nutrition, the chemistry and technology of cottonseed, and related fields.

Cottonseed is one of the principal plant materials being studied as a potential source of protein to help remedy the shortage of this nutritional element in the human diet. This phase of cottonseed utilization will be emphasized in the approaching conference.

The U. S. Department of Agriculture is co-sponsoring the meeting, through its Southern Utilization Research and Development Division, with the United Nations Children's Emergency Fund (UNICEF) and the National Cottonseed Products Association.

The meeting is open to those interested in this subject, Dr. C. H. Fisher, Director of the Southern Division, said in announcing the program. Those wishing to attend can obtain further information by writing Dr. B. H. Wojcik, Assistant Director, Southern Utilization Research and Development Division, P. O. Box 19687, New Orleans, La. 70119.

The Southern Division and the National Cottonseed Products Association are two of the world's leaders in research on the chemistry, processing and utilization of cottonseed, and have worked closely with UNICEF in efforts to develop a program for use of cottonseed protein as a dietary supplement for humans, especially children, in nations where protein supplies are insufficient.

Dr. A. M. Altschul, Chief Research Chemist of the Seed Protein Pioneering Research Laboratory of the Southern Division, is general chairman of the conference, with Dr. Wojcik and L. W. Mazzeno, Jr., also of the Southern Division, as cochairmen.

U. S. DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Southern Utilization Research and Development Division
New Orleans, Louisiana

FOR IMMEDIATE RELEASE January 29, 1964

World Conference on Cottonseed Protein Marks Industry Progress:

The brightest prospects for expansion of markets for cottonseed meals as well as other oilseed meals lies in increased utilization of these products for human consumption. The greatest commercial market potentials for such products are likely to be found in the industrial and high-income countries, even though interest in such products has been largely focused heretofore on the low-income and slowly developing countries.

A world conference on cottonseed protein concentrates held in New Orleans recently heard these reports on economic prospects for vegetable proteins, together with reviews on the fundamental, scientific, and technological advances in cottonseed investigations made since the previous conference held there in 1960. The conference was sponsored by the U. S. Department of Agriculture, through the Agricultural Research Service, Southern Utilization Research and Development Division, in cooperation with the United Nations Children's Fund (UNICEF), and the National Cottonseed Products Association.

The conference was attended by 87 research scientists, engineers, and representatives of various government agencies and commercial firms interested in the development and introduction of vegetable protein products for human nutrition. Participants were from the United States, Canada, Central America, Peru, India, Italy, Mexico, South Africa, and Turkey.

Dr. C. H. Fisher, Director of the Southern Division, welcomed the visitors and Dr. Ruth M. Leverton, Assistant Administrator of the Agricultural Research Service, Washington, D. C., and Dr. Grace A. Goldsmith, of the Tulane University Medical School gave opening remarks. Dr. Leverton represented Dr. George W. Irving, Jr., Deputy Administrator, ARS.

Dr. Aaron M. Altschul, Chief Research Chemist of the Seed Protein Pioneering Research Laboratory of the Southern Division, was general chairman for the conference and took the leading part in arranging the program.

The conference was dedicated to the memory of H. D. "Doc" Fincher, for many years a leader in the oilseed processing industry, who died in 1963. Dr. Altschul, in a formal tribute, said of him that ". . . . He had great responsibility for effecting these changes (in the cottonseed processing industry); it is because of people like him that cottonseed processing is a modern industry capable of turning out quality products and prepared to improve its operations through its technical know-how".

The prediction regarding the future of plant proteins in human nutrition was made by Dr. H. L. Wilcke, Vice President and Director of Research for the

Ralston-Purina Company, St. Louis. He pointed out that in 1947 meal accounted for 45.5 percent of the total value of soybeans, and 28 percent of the total value of cottonseed. In 1962-63, the value of soybean meal had increased to 63.7 percent of the total, and cottonseed meal to 40.5 percent.

Current usage figures, Dr. Wilcke said, show that practically all of the cottonseed and soybean meals are now going into animal feeds, but that animal and poultry feeds cannot continue to absorb more and more plant protein concentrates at favorable price levels. As one of the more spectacular demonstrations of the use of plant protein in the human diet, he cited the use of soy protein fibers in the production of foods with meatlike fibrous texture. The possibility of totally new food forms based on plant proteins is an attractive goal, he said, and a large amount of industrial research is now going on in this area.

P. H. Hind, of Hind Bros. Company, The Garden Factory, Durban, South Africa, illustrated the possibilities in the commercial introduction of such a protein supplement to the general public in his report on the marketing of their formulation called "ProNutro." The Hind firm first became interested in such a product through reports by a physician of the serious problems of malnutrition in native tribes in South Africa. Demand for the product has outstripped manufacturing capacity. The ProNutro formula depends largely upon peanut and soybean flour for its protein content, since these are the vegetable protein sources most readily available in that area.

Experiences in introducing and marketing food supplements with a high-protein content supplied by vegetable materials were also reported by Dr. H. W. Bruins, of the Quaker Oats Company Research Laboratory. He said that about four years ago they began exploring the marketing of Incaparina in Colombia, under an agreement with the Nutrition Institute of Central America and Panama, where they already had a plant and marketing organization, primarily with the idea of helping solve the severe malnutrition problem prevalent there. An intensive educational program and support of the medical profession and other workers in the field of health is necessary to success, he said, but great progress has been made in Colombia. He believes that a protein-rich product based partly on cottonseed flour can be sold there. He also said Quaker believes the time has come for commercial firms with production and marketing expertise to come to grips with the problem of protein lack in human diet, especially of children, and expressed the opinion that the Colombia operation should be "in the black" within three or four years.

Suitability of cottonseed protein for use in such a program, and technological advances in the production of a high-quality product were brought out in reports of several of the speakers. Notable among these were comments by Dr. Max T. Milner, UNICEF, on cottonseed flour produced in the pilot plant of the Southern Utilization Research and Development Division by the mixed solvent extraction process developed at the Southern Division by W. H. King and Dr. V. L. Frampton. He said that UNICEF workers had never before seen a processed cottonseed product with the nutritional quality of this flour, as indicated by the test for available lysine, and subsequently confirmed by animal feeding trials and metabolic studies of infant feeding. UNICEF workers were also impressed with the very light color of this product, in view of the fact that color is considered a major factor in general acceptability of the product.

The acetone extraction process employed by the G. & S. Vaccarino Company in Italy was described by Carmelo Vaccarino as producing a high-quality cottonseed meal. Mr. Vaccarino reported that young chickens used for biological trials grew best on rations in which the cottonseed meal was used in combination with soybean meal, but that the birds grew better on rations with cottonseed alone as a protein source than on an acceptable commercial ration. The cottonseed meal, Mr. Vaccarino said, is being sold in Italy at the same price as soybean meal of equal protein content, and the company is now planning to double plant capacity.

Stanley P. Clark, associate research engineer at the Cottonseed Products Research Laboratory, Texas A&M University, presented a flowsheet for the Vaccarino operation, and recommended that a detailed engineering study would be desirable before attempts were made to further commercial adaptation of the process.

The mixed-solvent extraction process is still in the pilot-plant stage, but E. A. Gastrock and H. L. E. Vix, of the Southern Division, reported that the process is sufficiently developed for serious consideration for commercial trials, especially in areas where cottonseed could be important as a source of protein concentrates. In this country, increased value of the products would be the critical factor.

For production of cottonseed protein for human nutrition in under-developed and low-income areas, Dr. Milner and other members of the UNICEF staff expressed the opinion that the ideal would be a food-grade operation providing about 10 tons of product per day, located adjacent to a conventional oilseed plant and operating in conjunction with it. It was generally agreed, however, that a great many scientific and technological problems in the utilization of cottonseed as a protein source are still unsolved, in spite of the advances made.

One section of the conference program was devoted to results of fundamental research on structure of the cottonseed and location in the cells of the various constituents. Mrs. Wilda H. Martinez, of the Southern Division, reported that various amino acids occur in different proportions in different parts of the cells. Dr. Altschul, in his summation of the conference, suggested that information of this kind might form the basis for an entirely new concept of processing methods in the future. As he put it, present methods of processing, by which the meats are first crushed and then separated into oil, protein, and other components, are like breaking and mixing packages of pennies, nickels, and quarters, then re-separating them according to denomination.

Charles F. Lewis, research geneticist with the Crops Research Division, USDA, told the conference that experimental strains of cotton have been developed without the darkly pigmented glands in the plants, and virtually no gossypol in the seed. Seed stocks and pollen have been made available to both public and private breeders. Many commercial breeders are now entering glandless strains in their performance trials, but are reluctant to forecast a date for the release of glandless varieties. Dr. A. B. Watts, head of the Poultry Science Department at Louisiana State University, reported favorably on results of feeding tests conducted with meals from the glandless seeds.

Surveying domestic and foreign market potentials for food protein products, Frank D. Barlow, Jr., Chief of the Export Programs Research Branch, Development and Trade Analysis Division of the Economic Research Service, USDA, and Wayne Bitting, Product and Process Evaluation Staff, Agricultural Research Service, USDA, said that world food needs exceed current as well as anticipated food availabilities. As estimated in the world food budget projections for 1966, relative nutritional deficiencies are: animal protein, in terms of nonfat milk solids, 1.5 million metric tons; pulse protein, in terms of dry beans and peas, 0.2 million metric tons; other protein and calories, in terms of wheat, 29.3 million metric tons; and fat, in terms of vegetable oil, 1.9 million metric tons. Not all of this deficit constitutes a commercial market, since much of it occurs in low-income, slowly developing countries, but cottonseed, soybean and fish appear to be the cheapest sources of protein.